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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	47675-14
First Inventor	Markl, Isabel et al.
Title	METHYLATION ALTERED DNA SEQUENCE
Express Mail Label No.	EL695285386US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. [ ] Fee Transmittal Form (e.g., PTO/SB/17) *(Submit an original and a duplicate for fee processing)*
2. [ ] Applicant claims small entity status. See 37 CFR 1.27.
3. [X] Specification [Total Pages 14]
   (preferred arrangement set forth below)
   - Descriptive title of the invention
   - Cross Reference to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to sequence listing, a table, or a computer program listing appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
4. [ ] Drawing(s) *(35 U.S.C. 113)* [Total Sheets [ ]]
5. Oath or Declaration [Total Pages [ ]]
   a. [ ] Newly executed (original or copy)
   b. [ ] Copy from a prior application (37 CFR 1.63 (d)) *(for continuation/divisional with Box 17 completed)*
      i. [ ] DELETION OF INVENTOR(S)
      Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1 63(d)(2) and 1.33(b).
6. [ ] Application Data Sheet. See 37 CFR 1.76
7. [ ] CD-ROM or CD-R in duplicate, large table or Computer Program *(Appendix)*
8. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. [X] Computer Readable Form (CRF)
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ACCOMPANYING APPLICATION PARTS

9. [ ] Assignment Papers (cover sheet & document(s))
10. [ ] 37 CFR 3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
11. [ ] English Translation Document *(if applicable)*
12. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
13. [ ] Preliminary Amendment
14. [X] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Markl, Isabel et al.

Filing Date: October 27, 2000

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For: METHYLATION ALTERED DNA SEQUENCES AS MARKERS ASSOCIATED WITH HUMAN CANCER

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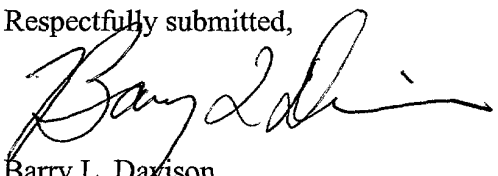

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METHYLATION ALTERED DNA SEQUENCES AS MARKERS ASSOCIATED WITH HUMAN CANCER

Technical Field of the Invention

The present invention relates to novel human DNA sequences that exhibit altered methylation patterns (hypermethylation or hypomethylation) in cancer patients. These novel methylation-altered DNA sequences are useful as diagnostic, prognostic and therapeutic markers for human cancer.

Background of the Invention

The identification of early genetic changes in tumorigenesis is a primary focus in molecular cancer research. Characterization of the nature and pattern of cancer-associated genetic alterations will allow for early detection, diagnosis and treatment of cancer. Such genetic alterations in vertebrates fall generally into one of three categories: gain or loss of genetic material; mutation of genetic material; or methylation at cytosine residues in CpG dinucleotides within "CpG islands." Among these, DNA methylation is uniquely reversible, and changes in methylation state are known to affect gene expression (*e.g.*, transcriptional initiation of genes where CpG islands located at or near the promoter region) or genomic stability.

Methylation of CpG dinucleotides within CpG islands. DNA, in higher order eukaryotic organisms, is methylated only at cytosine residues located 5' to guanosine residues in CpG dinucleotides. This covalent modification of the C-5 position of the cytosine base by the enzyme DNA (cytosine-5)-methyltransferase results in the formation of 5-methylcytosine (5-mCyt), and gives this base unique properties (*e.g.*, susceptibility to undergo spontaneous deamination). This enzymatic conversion is the only epigenetic modification of DNA known to exist in vertebrates, and is essential for normal embryonic development (Bird, A.P., *Cell* 70:5-8, 1992; Laird & Jaenisch, *Human Molecular Genetics* 3:1487-1495, 1994; Li et al., *Cell* 69:915-926, 1992).

The presence of 5-mCyt at CpG dinucleotides has resulted in the 5-fold depletion of this sequence in the genome during the course of vertebrate evolution (Schroderet & Gartler, *Proc. Nat. Acad. Sci. USA* 89:957-961, 1992), presumably due to spontaneous deamination of 5-mCyt to Thymidine. Certain areas of the genome, however, do not show such depletion, and are referred to as "CpG islands" (Bird, A.P., *Nature* 321:209-213, 1986; Gardiner-Garden & Frommer, *J. Mol. Biol.* 196:261-282, 1987). These CpG islands comprise only approximately 1% of the vertebrate genome, yet account for about 15% of the total number of genomic CpG dinucleotides (Antequera & Bird, *Proc. Nat. Acad. Sci. USA* 90:11995-11999, 1993). CpG islands contain the expected (*i.e.*, the non-evolutionarily depleted) frequency of

CpGs (with an Observed/Expected Ratio[1] >0.6), are GC-rich (with a GC Content[2] >0.5) and are typically between about 0.2 to about 1 kb in length.

Methylation within CpG islands affects gene expression. CpG islands are located upstream of many housekeeping and tissue-specific genes, but may also extend into gene coding regions (Cross & Bird, *Current Opinions in Genetics and Development* 5:309-314, 1995; Larsen et al., *Genomics* 13:1095-1107, 1992). The methylation of cytosines within CpG islands in somatic tissues is believed to affect gene expression. Methylation has been inversely correlated with gene activity and may lead to decreased gene expression by a variety of mechanisms including inhibition of transcription initiation (Bird, A.P., *Nature* 321:209-213, 1986; Delgado et al., *EMBO Journal* 17:2426-2435, 1998), disruption of local chromatin structure (Counts & Goodman, *Molecular Carcinogenesis* 11:185-188, 1994; Antequera et al., *Cell* 62:503-514, 1990), and recruitment of proteins that interact specifically with methylated sequences and thereby directly or indirectly prevent transcription factor binding (Bird, A.P., *Cell* 70:5-8, 1992; Counts & Goodman, *Molecular Carcinogenesis* 11:185-188, 1994; Cedar, H., *Cell* 53:3-4, 1988). Many studies have demonstrated the effect of methylation of CpG islands on gene expression (e.g., the *CDKN2A/p16* gene; Gonzalez-Zulueta et al., *Cancer Research* 55:4531-4535, 1995), but most CpG islands on autosomal genes remain unmethylated in the germline, and methylation of these islands is usually independent of gene expression. Tissue-specific genes are typically unmethylated in the respective target organs but are methylated in the germline and in non-expressing adult tissues, while CpG islands of constitutively expressed housekeeping genes are normally unmethylated in the germline and in somatic tissues.

Methylation within CpG islands affects the expression of genes involved in cancer. Data from a group of studies show the presence of altered methylation in cancer cells relative to non-cancerous cells. These studies show not only alteration of the overall genomic levels of DNA methylation, but also changes in the distribution of methyl groups. For example, abnormal methylation of CpG islands that are associated with tumor suppressor genes or oncogenes within a cell may cause altered gene expression. Such altered gene expression may provide a population of cells with a selective growth advantage and thereby result in selection of these cells to the detriment of the organism (*i.e.,* cancer).

Insufficient correlative data. Unfortunately, the mere knowledge of the basic existence of altered methylation of CpG dinucleotides within CpG islands of cancer cells relative to normal cells, or of the fact that in particular instances such methylation changes result in altered gene expression (or chromatin structure or stability), is inadequate to allow for effective diagnostic, prognostic and therapeutic application of this knowledge. This is

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[1] Calculated as: [number of CpG sites / (number of C bases X number of G bases)] X band length for each fragment.

[2] Calculated as: (number of C bases + number of G bases) / band length for each fragment.

because only a limited number of CpG islands have been characterized, and thus there is insufficient knowledge, as to which particular CpG islands, among many, are actually involved in, or show significant correlation with cancer or the etiology thereof. Moreover, complex methylation patterns, involving a plurality of methylation-altered DNA sequences, including those that may have the sequence composition to qualify as CpG islands, may exist in particular cancers.

Therefore there is a need in the art to identify and characterize specific methylation altered DNA sequences, and to correlate them with cancer to allow for their diagnostic, prognostic and therapeutic application.

Summary of the Invention

The present invention provides for a diagnostic or prognostic assay for cancer, comprising: obtaining a tissue sample from a test tissue; performing a methylation assay on DNA derived from the tissue sample, wherein the methylation assay determines the methylation state of a CpG dinucleotide within a DNA sequence of the DNA, and wherein the DNA sequence is a sequence selected from the group consisting of sequences of SEQ ID NOS:1-103, sequences having a nucleotide sequence at least 90% identical to sequences of SEQ ID NOS:1-103, CpG island sequences associated with sequences of SEQ ID NOS:1-103, CpG island sequences associated with sequences having a nucleotide sequence at least 90% identical to sequences of SEQ ID NOS:1-103, and combinations thereof, wherein the CpG island sequence associated with the sequence of the particular SEQ ID NO is that contiguous sequence of genomic DNA that encompasses at least one nucleotide of the particular SEQ ID NO sequence, and satisfies the criteria of having both a frequency of CpG dinucleotides corresponding to an Observed/Expected Ratio >0.6, and a GC Content >0.5; and determining a diagnosis or prognosis based, at least in part, upon the methylation state of the CpG dinucleotide within the DNA sequence. Preferably, the DNA sequence is a sequence selected from the group consisting of CpG island sequences associated with sequences of SEQ ID NOS:1-103, CpG island sequences associated with sequences having a nucleotide sequence at least 90% identical to sequences of SEQ ID NOS:1-103, and combinations thereof. Preferably, the DNA sequence is a sequence selected from the group consisting of CpG island sequences associated with sequences of SEQ ID NOS: 2, 4, 6, 7, 9-16, 19, 20, 22-33, 35-43, 48, 51-55, 59, 60, 64, 71, 76, 78-81, 84 and 87-90, and combinations thereof. Preferably, the methylation assay procedure is selected from the group consisting of MethyLight, MS-SnuPE (methylation-sensitive single nucleotide primer extension), MSP (methylation-specific PCR), MCA (methylated CpG island amplification), COBRA (combined bisulfite restriction analysis), and combinations thereof. Preferably, the methylation state of the CpG dinucleotide within the DNA sequence is that of hypermethylation, hypomethylation or normal methylation. Preferably, the cancer is selected

from the group consisting of bladder cancer, prostate cancer, colon cancer, lung cancer, renal cancer, leukemia, breast cancer, uterine cancer, astrocytoma, glioblastoma, and neuroblastoma. Preferably, the cancer is bladder cancer, or prostate cancer.

The present invention further provides a kit useful for the detection of a methylated CpG-containing nucleic acid comprising a carrier means containing one or more containers comprising: a container containing a probe or primer which hybridizes to any region of a sequence selected from the group consisting of SEQ ID NOS:1-103, and sequences having a nucleotide sequence at least 90% identical to sequences of SEQ ID NOS:1-103; and additional standard methylation assay reagents required to affect detection of methylated CpG-containing nucleic acid based on the probe or primer. Preferably, the additional standard methylation assay reagents are standard reagents for performing a methylation assay from the group consisting of MethyLight, MS-SNuPE, MSP, MCA, COBRA, and combinations thereof. Preferably, the probe or primer comprises at least about 12 to 15 nucleotides of a sequence selected from the group consisting of SEQ ID NOS:1-103, and sequences having a nucleotide sequence at least 90% identical to sequences of SEQ ID NOS:1-103.

The present invention further provides an isolated nucleic acid molecule comprising a methylated or unmethylated polynucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:97, and SEQ ID NO:100. Preferably the nucleic acid is methylated. Preferably, the nucleic acid is unmethylated.

Detailed Description of the Invention

Definitions:

"GC Content" refers, within a particular DNA sequence, to the [(number of C bases + number of G bases) / band length for each fragment].

"Observed/Expected Ratio" ("O/E Ratio") refers to the frequency of CpG dinucleotides within a particular DNA sequence, and corresponds to the [number of CpG sites / (number of C bases X number of G bases)] X band length for each fragment.

"CpG Island" refers to a contiguous region of genomic DNA that satisfies the criteria of (1) having a frequency of CpG dinucleotides corresponding to an "Observed/Expected Ratio" >0.6), and (2) having a "GC Content" >0.5. CpG islands are

typically, but not always, between about 0.2 to about 1 kb in length. A CpG island sequence associated with a particular SEQ ID NO sequence of the present invention is that contiguous sequence of genomic DNA that encompasses at least one nucleotide of the particular SEQ ID NO sequence, and satisfies the criteria of having both a frequency of CpG dinucleotides corresponding to an Observed/Expected Ratio >0.6), and a GC Content >0.5.

"Methylation state" refers to the presence or absence of 5-methylcytosine ("5-mCyt") at one or a plurality of CpG dinucleotides within a DNA sequence.

"Hypermethylation" refers to the methylation state corresponding to an *increased* presence of 5-mCyt at one or a plurality of CpG dinucleotides within a DNA sequence of a test DNA sample, relative to the amount of 5-mCyt found at corresponding CpG dinucleotides within a normal control DNA sample.

"Hypomethylation" refers to the methylation state corresponding to a *decreased* presence of 5-mCyt at one or a plurality of CpG dinucleotides within a DNA sequence of a test DNA sample, relative to the amount of 5-mCyt found at corresponding CpG dinucleotides within a normal control DNA sample.

"Methylation assay" refers to any assay for determining the methylation state of a CpG dinucleotide within a sequence of DNA.

"MS.AP-PCR" (**M**ethylation-**S**ensitive **A**rbitrarily-**P**rimed **P**olymerase **C**hain **R**eaction) refers to the art-recognized technology that allows for a global scan of the genome using CG-rich primers to focus on the regions most likely to contain CpG dinucleotides, and described by Gonzalgo et al., *Cancer Research* 57:594-599, 1997.

"MethyLight" refers to the art-recognized fluorescence-based real-time PCR technique described by Eads et al., *Cancer Res.* 59:2302-2306, 1999.

"Ms-SNuPE" (**M**ethylation-sensitive **S**ingle **Nu**cleotide **P**rimer **E**xtension) refers to the art-recognized assay described by Gonzalgo & Jones, *Nucleic Acids Res.* 25:2529-2531, 1997.

"MSP" (**M**ethylation-**s**pecific **P**CR) refers to the art-recognized methylation assay described by Herman et al. *Proc. Natl. Acad. Sci. USA* 93:9821-9826, 1996, and by US Patent No. 5,786,146.

"COBRA" (**Co**mbined **Bi**sulfite **R**estriction **A**nalysis) refers to the art-recognized methylation assay described by Xiong & Laird, *Nucleic Acids Res.* 25:2532-2534, 1997.

"MCA" (**M**ethylated **C**pG Island **A**mplification) refers to the methylation assay described by Toyota et al., *Cancer Res.* 59:2307-12, 1999, and in WO 00/26401A1.

Overview

The present invention provides for 103 DNA sequences (*i.e.*, "marker sequences") having distinct methylation patterns in cancer, as compared to normal tissue. These methylation-altered DNA sequence embodiments correspond to 103 DNA fragments isolated

from bladder and prostate cancer patients, and in many instances, represent novel sequences not found in the GenBank database. *None* of the instant sequence embodiments have previously been characterized with respect to their methylation pattern in human cancers including, but not limited to, those of the bladder and prostate. The significance of such methylation patterns lies in the value of altered fragments as potential prognostic, diagnostic and therapeutic markers in the treatment of human cancers.

Identification of Methylation-altered Marker Sequences in Genomic DNA

The MS.AP-PCR technique was used to scan the genomes of bladder or prostate cancer patients for DNA methylation changes relative to normal individuals, because the pattern is known to be highly conserved. A total of 103 DNA sequence embodiments (methylation-altered DNA sequences; "marker sequences") were isolated and characterized as having distinct methylation patterns in cancer, as compared to normal tissue.

Methods for the Identification of Marker Sequences in Genomic DNA. There are a variety of art-recognized genome scanning methods that have been used to identify altered methylation sites in cancer cells. For example, one method involves restriction landmark genomic scanning (Kawai et al., *Mol. Cell. Biol.* 14:7421-7427, 1994), another involves MCA (methylated CpG island amplification; Toyota et al., *Cancer Res.* 59:2307-12, 1999), and yet another involves MS.AP-PCR (Methylation-Sensitive Arbitrarily-Primed Polymerase Chain Reaction; Gonzalgo et al., *Cancer Res.* 57:594-599, 1997), which allows for a global scan of the genome using CG-rich primers to focus on the regions most likely to contain CpG dinucleotides. The MS.AP-PCR technique used in the present invention is a rapid and efficient method to screen ("scan") for altered methylation patterns in genomic DNA and to isolate specific sequences associated with these changes.

Briefly, genomic DNA from the tissue of bladder or prostate cancer patients was prepared using standard, art-recognized methods. Restriction enzymes (*e.g.*, HpaII) with different sensitivities to cytosine methylation in their recognition sites were used to digest these genomic DNAs prior to arbitrarily primed PCR amplification with GC-rich primers. Fragments that showed differential methylation (*e.g.*, *hypermethylation* or *hypomethylation*, based on the methylation sensitivity of the restriction enzyme, or upon DNA sequence analysis or Ms-SNuPE analysis; Gonzalgo & Jones, *Nucleic Acids Res* 25:2529-2531, 1997) were cloned and sequenced after resolving the PCR products on high-resolution polyacrylamide gels. The cloned fragments were used as probes for Southern blot analysis to confirm differential methylation of these regions in the tissue. Methods for DNA cloning, sequencing, PCR, high-resolution polyacrylamide gel resolution and Southern blot analysis are well known by those of ordinary skill in the relevant art.

Results. A total of 500 DNA fragments that underwent either hypermethylation (an increase in the level of methylation relative to normal) or hypomethylation (a decrease in the

level of methylation relative to normal) were isolated from the scanned patients genomic DNA. A total of 178 of these fragments were sequenced, of which 103 were *novel* in that they corresponded to DNA loci whose methylation pattern had not previously been characterized. The corresponding sequences are disclosed as [SEQ ID NOS:1-103], wherein for certain sequences, the letter "n" refers to an undetermined nucleotide base.

Novel marker sequences identified by MS.AP-PCR. Table I shows an *overall* summary of methylation patterns and sequence data corresponding to the 103 DNA fragments identified by MS.AP-PCR. A total of 103 fragments were sequenced following identification as becoming either hypermethylated (gain of methylation; noted as having a hypermethylation pattern) or hypomethylated (loss of methylation; noted as having a hypomethylation pattern) relative to normal tissue. For the fragments of each category, the "Average GC Content" is shown, calculated as (number of C bases + number of G bases)/band length for each fragment, as well as the average Observed/Expected Ratio ("O/E Ratio"), calculated as [number of CpG sites/(number of C bases X number of G bases)] X band length for each fragment. Additionally, the percent of fragments that qualify as CpG islands is listed, and corresponds to the percentage of all fragments within each category that have sequence compositions that satisfy the criteria of having a "GC Content" >0.5 and an "O/E Ratio" >0.6.

Thus, of these 103 fragments identified by MS.AP-PCR, 60 showed hypermethylation (Table I, upper row; Table II, [SEQ ID NOS:1-60]) while 43 showed hypomethylation (Table I, lower row; Table II, [SEQ ID NOS:61-103]). Moreover, 55 (43 hypermethylated, and 12 hypomethylated) of the 103 fragments correspond to CpG islands (*i.e.*, fulfill the criteria of a GC content >0.5 and an Observed/Expected Ratio >0.6;), whereas the other 48 (17 hypermethylated and 31 hypomethylated) fragments do not meet the criteria for CpG islands (*see* Table II).

TABLE I. Summary of 103 DNA Fragments Identified by MS.AP-PCR

DNA Fragment Type	Methylation Pattern (relative to normal)	Number of Fragments (103 total)	Average GC Content	Average O/E Ratio	Percent that correspond to CpG Islands
Hypermethylated Fragments	Hyper-methylation	60	0.54	0.72	72%
Hypomethylated Fragments	Hypo-methylation	43	0.52	0.48	28%

Table II shows a summary of methylation pattern and sequence data for each *individual* sequence embodiment ([SEQ ID NOS:1-103]), corresponding to the 103 DNA fragments identified by MS.AP-PCR. Data for the 103 fragments was divided into either hypermethylated ([SEQ ID NOS:1-60]) or hypomethylated ([SEQ ID NOS:61-103]) categories. Table II also lists, for each sequence embodiment, the corresponding "Fragment

Name," fragment "Size" (in base pairs; "bp"), "GC Content," Observed/Expected Ratio ("O/E Ratio"), "Description" (*i.e.*, as a CpG island if criteria are met), "Inventor Initials" (IDCM = Isabel D.C. Markl, JC = Jonathan Cheng, GL = Gangning Liang, HF = Hualin Fu, YT = Yoshitaka Tomigahara), "Cancer Source," and "Chromosome Match" to the GenBank database. A dash ("-") indicates that no GenBank chromosome match existed, or that only a low-scoring partial match was found. Averages of the "GC Content" and "O/E Ratio," along with the percent of fragments that are CpG islands, are listed after the last member of both the hypermethylated and hypomethylated categories.

Therefore, the present invention provides for 103 DNA fragments and corresponding marker sequence embodiments (*i.e.*, methylation-altered DNA sequences) that are useful in cancer prognostic, diagnostic and therapeutic applications.

Additionally, at least 55 of these 103 sequences correspond to CpG islands (based on GC Content and O/E ration); namely [SEQ ID NOS:2, 4, 6, 7, 9-16, 19, 20, 22-33, 35-43, 48, 51-55, 59, 60, 64, 71, 76, 78-81, 84 and 87-90]. Thus, based on the fact that the methylation state of a portion of a given CpG island is generally representative of the island as a whole, the present invention further encompassed the novel use of the 55 CpG islands associated with [SEQ ID NOS:2, 4, 6, 7, 9-16, 19, 20, 22-33, 35-43, 48, 51-55, 59, 60, 64, 71, 76, 78-81, 84 and 87-90] in cancer prognostic, diagnostic and therapeutic applications, where a CpG island sequence associated with the sequence of a particular SEQ ID NO is that contiguous sequence of genomic DNA that encompasses at least one nucleotide of the particular SEQ ID NO sequence, and satisfies the criteria of having both a frequency of CpG dinucleotides corresponding to an Observed/Expected Ratio >0.6, and a GC Content >0.5.

TABLE II. Summary of MS.AP-PCR Fragments Sequenced

Methylation Pattern	Fragment Name	Size (bp)	GC Content	O/E Ratio	Description	Inventor Initials	Cancer Source	Chromosome Matches	[SEQ ID NO]
Hyper-methylation category	11-1A	510	0.44	0.74		IDCM	Bladder	-	1
	14-3B	313	0.58	0.74	CpG Island	IDCM	Bladder	2	2
	18-2B	165	0.57	0.45		IDCM	Bladder	7	3
	24-1B	601	0.51	0.72	CpG Island	IDCM	Bladder	Xp11	4
	26-1B	801	0.48	0.56		IDCM	Bladder	-	5
	26-2C	204	0.50	0.63	CpG Island	IDCM	Bladder	-	6
	30-3D	205	0.55	1.25	CpG Island	IDCM	Bladder	14	7
	32-3E	597	0.57	0.10		IDCM	Bladder	20q12-13.1	8
	34-2B	500	0.62	0.66	CpG Island	IDCM	Bladder	20	9
	34-4B	343	0.70	0.81	CpG Island	IDCM	Bladder	-	10
	34-5D	291	0.62	0.96	CpG Island	IDCM	Bladder	9	11
	34-6A	266	0.64	0.93	CpG Island	IDCM	Bladder	-	12
	35-1C	553	0.64	0.63	CpG Island	IDCM	Bladder	-	13
	36-2D	156	0.60	0.58	CpG Island	IDCM	Bladder	10	14
	38-1A	300	0.70	0.80	CpG Island	IDCM	Bladder	10	15
	38-2B	196	0.56	0.89	CpG Island	IDCM	Bladder	15	16
	7-8E	299	0.59	0.39		IDCM	Bladder	17q21-22	17
	83-4B	363	0.54	0.49		IDCM	Bladder	-	18

Methylation Pattern	Fragment Name	Size (bp)	GC Content	O/E Ratio	Description	Inventor Initials	Cancer Source	Chromosome Matches	[SEQ ID NO]
	84-1D	322	0.55	0.90	CpG Island	IDCM	Bladder	7	19
	101-3E	255	0.57	0.83	CpG Island	IDCM	Bladder	17	20
	M1-5A	406	0.45	0.96		IDCM	Bladder	1	21
	U2-8E	210	0.56	0.61	CpG Island	IDCM	Bladder	2	22
	U12-1A	310	0.56	0.81	CpG Island	IDCM	Bladder	2	23
	U7-4A	305	0.59	0.80	CpG Island	IDCM	Bladder	-	24
	NU9-5A	379	0.67	0.83	CpG Island	JC	Bladder	-	25
	3-17-8-B	625	0.48	0.72	CpG Island	GL	Bladder	18	26
	4-10-4-A	499	0.55	0.30	CpG Island	GL	Bladder	7	27
	1-1-1-A	561	0.58	0.98	CpG Island	GL	Bladder	20	28
	3-17-8-A	717	0.50	0.68	CpG Island	GL	Bladder	17	29
	G145-H	280	0.50	1.10	CpG Island	GL	Bladder	11	30
	1-1-1-D	270	0.50	0.60	CpG Island	GL	Bladder	2	31
	1-1-1-C	347	0.65	1.25	CpG Island	GL	Bladder	-	32
	G178-A	342	0.55	0.85	CpG Island	GL	Bladder	2	33
	34-A	370	0.62	0.44		HF	Prostate	-	34
	34-D	213	0.53	0.74	CpG Island	HF	Prostate	2	35
	35-D	173	0.56	0.66	CpG Island	HF	Prostate	3	36
	36-A	369	0.67	0.70	CpG Island	HF	Prostate	-	37
	40-A	123	0.60	1.16	CpG Island	HF	Prostate	-	38
	91-1	450	0.64	0.86	CpG Island	YT	Bladder	5 or 16q24.3	39
	93-2	593	0.51	0.68	CpG Island	YT	Bladder	Xp11	40
	93-3	457	0.52	0.94	CpG Island	YT	Bladder	Xp22.1-22.3	41
	94-8	211	0.66	0.96	CpG Island	YT	Bladder	-	42
	95-5	141	0.63	0.79	CpG Island	YT	Bladder	14	43
	97-5	559	0.56	0.40		YT	Bladder	-	44
	98-1	433	0.46	0.96		YT	Bladder	1	45
	100-1	487	0.59	0.58		YT	Bladder	14	46
	100-2	403	0.60	0.47		YT	Bladder	3	47
	100-6	155	0.57	0.99	CpG Island	YT	Bladder	20	48
	4-2	256	0.57	0.40		YT	Bladder	7	49
	5-8	224	0.47	0.96		YT	Bladder	5	50
	6-4	313	0.70	0.82	CpG Island	YT	Bladder	-	51
	7-6	385	0.70	0.88	CpG Island	YT	Bladder	-	52
	13-3	307	0.59	0.89	CpG Island	YT	Bladder	10	53
	15-2	182	0.62	0.92	CpG Island	YT	Bladder	13	54
	23-2	523	0.54	0.87	CpG Island	YT	Bladder	Xp22.1-22.3	55
	39-2	795	0.46	0.64		YT	Bladder	13	56
	40-2	438	0.62	0.51		YT	Bladder	10	57
	41-3	611	0.47	0.70		YT	Bladder	18	58
	105-4	291	0.58	0.71	CpG Island	YT	Bladder	5	59
	107-8	226	0.53	0.96	CpG Island	YT	Bladder	11	60
AVERAGE			*0.54*	*0.72*	*72% islands*				
Hypo-methylation category	14-2B	580	0.55	0.51		IDCM	Bladder	2	61
	16-1B	633	0.56	0.39		IDCM	Bladder	-	62
	18-1B	703	0.45	0.35		IDCM	Bladder	17	63
	19-1B	420	0.66	0.87	CpG Island	IDCM	Bladder	-	64
	20-1B	496	0.61	0.59		IDCM	Bladder	-	65
	21-2C	637	0.60	0.33		IDCM	Bladder	9q34	66
	29-1A	595	0.55	0.27		IDCM	Bladder	Xp11.23	67
	29-2B	580	0.47	0.77		IDCM	Bladder	-	68
	32-1A	589	0.59	0.48		IDCM	Bladder	-	69
	34-1B	450	0.42	0.46		IDCM	Bladder	-	70
	34-3B	432	0.70	0.61	CpG Island	IDCM	Bladder	-	71

Methylation Pattern	Fragment Name	Size (bp)	GC Content	O/E Ratio	Description	Inventor Initials	Cancer Source	Chromosome Matches	[SEQ ID NO]
	32-2B	748	0.47	0.24		IDCM	Bladder	2	72
	32-4B	599	0.57	0.15		IDCM	Bladder	20q12-13.1	73
	32-5B	614	0.58	0.20		IDCM	Bladder	-	74
	33-1A	552	0.54	0.32		IDCM	Bladder	10	75
	5-1E	501	0.61	1.04	CpG Island	IDCM	Bladder	-	76
	6-1A	826	0.55	0.36		IDCM	Bladder	22q13.32-13.33	77
	7-5D	433	0.59	0.85	CpG Island	IDCM	Bladder	5	78
	8-7C	424	0.58	0.83	CpG Island	IDCM	Bladder	5	79
	30-6D	285	0.63	0.72	CpG Island	IDCM	Bladder	1	80
	66-2E	401	0.54	0.82	CpG Island	IDCM	Bladder	16	81
	78-1C	268	0.54	0.41		IDCM	Bladder	-	82
	97-2E	989	0.53	0.16		IDCM	Bladder	-	83
	M1-8C	250	0.64	0.99	CpG Island	IDCM	Bladder	-	84
	M2-5A	402	0.50	0.45		IDCM	Bladder	5	85
	M1-4P	595	0.43	0.41		IDCM	Bladder	-	86
	M12-10A	304	0.53	0.76	CpG Island	IDCM	Bladder	7	87
	M12-12C	296	0.51	0.64	CpG Island	IDCM	Bladder	17	88
	M2-8M	220	0.67	0.62	CpG Island	IDCM	Bladder	6q27	89
	NU4-3A	273	0.63	1.02	CpG Island	JC	Bladder	-	90
	NU5-2A	361	0.44	0.73		JC	Bladder	6q14.3-15	91
	88-5	462	0.62	0.39		YT	Bladder	-	92
	90-1	591	0.66	0.45		YT	Bladder	19	93
	91-3	279	0.58	0.45		YT	Bladder	5 or 16q24.3	94
	91-4	351	0.55	0.30		YT	Bladder	18q23	95
	91-7	171	0.61	0.59		YT	Bladder	11	96
	89-3	743	0.55	0.43		YT	Bladder	-	97
	94-2	589	0.53	0.41		YT	Bladder	22q13.31-13.32	98
	94-3	538	0.53	0.49		YT	Bladder	5 or 18	99
	94-4	486	0.61	0.57		YT	Bladder	-	100
	94-5	450	0.60	0.45		YT	Bladder	1p36.2-36.3	101
	94-6	292	0.58	0.32		YT	Bladder	8 or 9	102
	96-4	395	0.63	0.54		YT	Bladder	9	103
AVERAGE			*0.52*	*0.48*	*28% islands*				

Diagnostic and Prognostic Assays for Cancer. The present invention provides for diagnostic and prognostic cancer assays based on determination of the methylation state of one or more of the disclosed 103 methylation-altered DNA sequence embodiments. Typically, such assays involve obtaining a tissue sample from a test tissue, performing a methylation assay on DNA derived from the tissue sample, and making a diagnosis or prognosis based thereon.

The methylation assay is used to determine the methylation state of one or a plurality of CpG dinucleotide within a DNA sequence of the DNA sample. According to the present invention, possible methylation states include *hypermethylation* and *hypomethylation*, relative to a normal state (*i.e.*, non-cancerous control state). Hypermethylation and hypomethylation refer to the methylation states corresponding to an *increased* or *decreased*, respectively,

presence 5-methylcytosine ("5-mCyt") at one or a plurality of CpG dinucleotides within a DNA sequence of the test sample, relative to the amount of 5-mCyt found at corresponding CpG dinucleotides within a normal control DNA sample.

A diagnosis or prognosis is based, at least in part, upon the determined methylation state of the sample DNA sequence compared to control data obtained from normal, non-cancerous tissue.

Methylation Assay Procedures. Various methylation assay procedures are known in the art, and can be used in conjunction with the present invention. These assays allow for determination of the methylation state of one or a plurality of CpG dinucleotides (*e.g.*, CpG islands) within a DNA sequence. Such assays involve, among other techniques, DNA sequencing of bisulfite-treated DNA, PCR (for sequence-specific amplification), Southern blot analysis, use of methylation-sensitive restriction enzymes, etc.

For example, genomic sequencing has been simplified for analysis of DNA methylation patterns and 5-methylcytosine distribution by using bisulfite treatment (Frommer et al., *Proc. Natl. Acad. Sci. USA* 89:1827-1831, 1992). Additionally, restriction enzyme digestion of PCR products amplified from bisulfite-converted DNA is used, *e.g.*, the method described by Sadri & Hornsby (*Nucl. Acids Res.* 24:5058-5059, 1996), or COBRA (Combined Bisulfite Restriction Analysis) (Xiong & Laird, *Nucleic Acids Res.* 25:2532-2534, 1997).

COBRA. COBRA analysis is a quantitative methylation assay useful for determining DNA methylation levels at specific gene loci in small amounts of genomic DNA (Xiong & Laird, *Nucleic Acids Res.* 25:2532-2534, 1997). Briefly, restriction enzyme digestion is used to reveal methylation-dependent sequence differences in PCR products of sodium bisulfite-treated DNA. Methylation-dependent sequence differences are first introduced into the genomic DNA by standard bisulfite treatment according to the procedure described by Frommer et al. (*Proc. Natl. Acad. Sci. USA* 89:1827-1831, 1992). PCR amplification of the bisulfite converted DNA is then performed using primers specific for the interested CpG islands, followed by restriction endonuclease digestion, gel electrophoresis, and detection using specific, labeled hybridization probes. Methylation levels in the original DNA sample are represented by the relative amounts of digested and undigested PCR product in a linearly quantitative fashion across a wide spectrum of DNA methylation levels. In addition, this technique can be reliably applied to DNA obtained from microdissected paraffin-embedded tissue samples. Typical reagents (*e.g.*, as might be found in a typical COBRA-based kit) for COBRA analysis may include, but are not limited to: PCR primers for specific gene (or methylation-altered DNA sequence or CpG island); restriction enzyme and appropriate buffer; gene-hybridization oligo; control hybridization oligo; kinase labeling kit for oligo probe; and radioactive nucleotides. Additionally, bisulfite conversion reagents may include: DNA denaturation buffer; sulfonation buffer; DNA recovery regents or kit (*e.g.*,

precipitation, ultrafiltration, affinity column); desulfonation buffer; and DNA recovery components.

Preferably, assays such as "MethyLight" (a fluorescence-based real-time PCR technique) (Eads et al., *Cancer Res.* 59:2302-2306, 1999), Ms-SNuPE (Methylation-sensitive Single Nucleotide Primer Extension) reactions (Gonzalgo & Jones, *Nucleic Acids Res.* 25:2529-2531, 1997), methylation-specific PCR ("MSP"; Herman et al., *Proc. Natl. Acad. Sci. USA* 93:9821-9826, 1996; US Patent No. 5,786,146), and methylated CpG island amplification ("MCA";Toyota et al., *Cancer Res.* 59:2307-12, 1999) are used alone or in combination with other of these methods.

MethyLight. The MethyLight assay is a high-throughput quantitative methylation assay that utilizes fluorescence-based real-time PCR (TaqMan ®) technology that requires no further manipulations after the PCR step (Eads et al., *Cancer Res.* 59:2302-2306, 1999). Briefly, the MethyLight process begins with a mixed sample of genomic DNA that is converted, in a sodium bisulfite reaction, to a mixed pool of methylation-dependent sequence differences according to standard procedures (the bisulfite process converts unmethylated cytosine residues to uracil). Fluorescence-based PCR is then performed either in an "unbiased" (with primers that do not overlap known CpG methylation sites) PCR reaction, or in a "biased" (with PCR primers that overlap known CpG dinucleotides) reaction. Sequence discrimination can occur either at the level of the amplification process or at the level of the fluorescence detection process, or both.

The MethyLight may assay be used as a quantitative test for methylation patterns in the genomic DNA sample, wherein sequence discrimination occurs at the level of probe hybridization. In this quantitative version, the PCR reaction provides for unbiased amplification in the presence of a fluorescent probe that overlaps a particular putative methylation site. An unbiased control for the amount of input DNA is provided by a reaction in which neither the primers, nor the probe overlie any CpG dinucleotides. Alternatively, a qualitative test for genomic methylation is achieved by probing of the biased PCR pool with either control oligonucleotides that do not "cover" known methylation sites (a fluorescence-based version of the "MSP" technique), or with oligonucleotides covering potential methylation sites.

The MethyLight process can by used with a "TaqMan®" probe in the amplification process. For example, double-stranded genomic DNA is treated with sodium bisulfite and subjected to one of two sets of PCR reactions using TaqMan® probes; *e.g.*, with either biased primers and TaqMan® probe, or unbiased primers and TaqMan® probe. The TaqMan® probe is dual-labeled with fluorescent "reporter" and "quencher" molecules, and is designed to be specific for a relatively high GC content region so that it melts out at about 10 °C higher temperature in the PCR cycle than the forward or reverse primers. This allows the TaqMan® probe to remain fully hybridized during the PCR annealing/extension step. As the

Taq polymerase enzymatically synthesizes a new strand during PCR, it will eventually reach the annealed TaqMan® probe. The Taq polymerase 5' to 3' endonuclease activity will then displace the TaqMan® probe by digesting it to release the fluorescent reporter molecule for quantitative detection of its now unquenched signal using a real-time fluorescent detection system.

Typical reagents (*e.g.*, as might be found in a typical MethyLight-based kit) for MethyLight analysis may include, but are not limited to: PCR primers for specific gene (or methylation-altered DNA sequence or CpG island); TaqMan® probes; optimized PCR buffers and deoxynucleotides; and Taq polymerase.

Ms-SNuPE. The Ms-SNuPE technique is a quantitative method for assessing methylation differences at specific CpG sites based on bisulfite treatment of DNA, followed by single-nucleotide primer extension (Gonzalgo & Jones, *Nucleic Acids Res.* 25:2529-2531, 1997). Briefly, genomic DNA is reacted with sodium bisulfite to convert unmethylated cytosine to uracil while leaving 5-methylcytosine unchanged. Amplification of the desired target sequence is then performed using PCR primers specific for bisulfite-converted DNA, and the resulting product is isolated and used as a template for methylation analysis at the CpG site(s) of interest. Small amounts of DNA can be analyzed (*e.g.*, microdissected pathology sections), and it avoids utilization of restriction enzymes for determining the methylation status at CpG sites. Typical reagents (*e.g.*, as might be found in a typical Ms-SNuPE-based kit) for Ms-SNuPE analysis may include, but are not limited to: PCR primers for specific gene (or methylation-altered DNA sequence or CpG island); optimized PCR buffers and deoxynucleotides; gel extraction kit; positive control primers; Ms-SNuPE primers for specific gene; reaction buffer (for the Ms-SNuPE reaction); and radioactive nucleotides. Additionally, bisulfite conversion reagents may include: DNA denaturation buffer; sulfonation buffer; DNA recovery regents or kit (*e.g.*, precipitation, ultrafiltration, affinity column); desulfonation buffer; and DNA recovery components.

MSP. MSP (methylation-specific PCR) allows for assessing the methylation status of virtually any group of CpG sites within a CpG island, independent of the use of methylation-sensitive restriction enzymes (Herman et al. *Proc. Natl. Acad. Sci. USA* 93:9821-9826, 1996; US Patent No. 5,786,146). Briefly, DNA is modified by sodium bisulfite converting all unmethylated, but not methylated cytosines to uracil, and subsequently amplified with primers specific for methylated versus unmethylated DNA. MSP requires only small quantities of DNA, is sensitive to 0.1% methylated alleles of a given CpG island locus, and can be performed on DNA extracted from paraffin-embedded samples. Typical reagents (*e.g.*, as might be found in a typical MSP-based kit) for MSP analysis may include, but are not limited to: methylated and unmethylated PCR primers for specific gene (or methylation-altered DNA sequence or CpG island), optimized PCR buffers and deoxynucleotides, and specific probes.

MCA. The MCA technique is a method that can be used to screen for altered methylation patterns in genomic DNA, and to isolate specific sequences associated with these changes (Toyota et al., *Cancer Res.* 59:2307-12, 1999). Briefly, restriction enzymes with different sensitivities to cytosine methylation in their recognition sites are used to digest genomic DNAs from primary tumors, cell lines, and normal tissues prior to arbitrarily primed PCR amplification. Fragments that show differential methylation are cloned and sequenced after resolving the PCR products on high-resolution polyacrylamide gels. The cloned fragments are then used as probes for Southern analysis to confirm differential methylation of these regions. Typical reagents (*e.g.*, as might be found in a typical MCA -based kit) for MCA analysis may include, but are not limited to: PCR primers for arbitrary priming Genomic DNA; PCR buffers and nucleotides, restriction enzymes and appropriate buffers; gene-hybridization oligos or probes; control hybridization oligos or probes.

Kits for Detection of Methylated CpG-containing Nucleic Acid. The reagents required to perform one or more art-recognized methylation assays (including those identified above) are combined with primers or probes comprising the sequences of SEQ ID NOS:1-103, or portions thereof, to determine the methylation state of CpG-containing nucleic acids. For example, the MethyLight, Ms-SNuPE, MCA, COBRA, and MSP methylation assays could be used alone or in combination, along with primers or probes comprising the sequences of SEQ ID NOS:1-103, or portions thereof, to determine the methylation state of a CpG dinucleotide within a genomic sequence corresponding to SEQ ID NOS:1-103, or to CpG island sequences associated with sequences of SEQ ID NOS:1-103, where the CpG island sequence associated with the sequence of the particular SEQ ID NO is that contiguous sequence of genomic DNA that encompasses at least one nucleotide of the particular SEQ ID NO sequence, and satisfies the criteria of having both a frequency of CpG dinucleotides corresponding to an Observed/Expected Ratio >0.6, and a GC Content >0.5.

SEQUENCE LISTING

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Tomigahara, Yoshitaka
Liang, Gangning
Fu, Hualin
Jones, Peter

<120> Methylation Altered DNA Sequences as Markers Associated with Human Cancer

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caaggaccgc gcgcgcgaag gcgccgtagc aagtgggcac acaccagaca ccaccccggc 120

gtgttccgcg ggagaagcca gtgcacacat cctcccgcaa ggcggggttg ccagtgcaac 180

acaggaatcc tgccctttt ctagaaaagc cccctccccc actttccctc caatacactc 240

acctgcgtct caacagtttc cttcttgcgc tacacgcggc cgctaagccg a 291

<210> 12
<211> 266
<212> DNA
<213> Homo sapiens

<400> 12
gtccggatca gtttccccgg ccaggtcgct tcccggtctc aaccatttcg cgctctgctc 60

tgtccgctgg tttgtccctg cccggttcct ctccccgggc ctgtcagcct ccgcttctct 120

ggaggttcct gggactcatc tctgatccac cgtcttgcgt tctctgggcg catcgacttc 180

tctccatctt cgggctcact cctgactccc tcgctgccgc ccccgggggt ttccacgcgt 240

gtctctaacc gcggccgcta agccga 266

<210> 13
<211> 553
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()

<223> "n" refers to an undetermined base

<400> 13
gatcctggtc catcgaaacc ttgtgtgcat cggttagtgc ttcctgggcg tttgcttcta 60

gccgacgctg acagtggagt gccagaaaga gggagaggac cgtcatggct actctgcccc 120

tggtgtcacc atgcgctctc ccccggcacc ggcgaggcga aacgtttcgc tagtccccgg 180

gaggcccctc ggtcagggca gcagcatccc tgcaccctct ccgcaggtgg tctccccgac 240

gccacaggtg gccagcaggg cgcgggtggg ggcaggagcg cctctcccct gcccaggcct 300

cccgctcctt ctcggagcgc tgtggcgggg tggagagaca gccttctaca gctagtctag 360

ctcggcgcgg ttcccgtctg tggcctccta atcccacagc cacagcgcct tcctctaacc 420

tccctcggtg ggcttaaagc ctcccgttcc ttctgtctca ttccttctgc tccctccccc 480

cgaaaccccc agatganagc tgggaacctg gcnccantna ctgagcnaac agtgttgacg 540

ggccgnggcc caa 553

<210> 14
<211> 156
<212> DNA
<213> Homo sapiens

<400> 14
gcgcacacag tgggtacaag gatgagctcg gtgtaaggaa tggaaagccc ccagtctaaa 60

ccaccgcccc ctagacacgg gtgaaaacct gcctaaaagc taactcaggc agtgactcta 120

tcacccgaag gggccctggg ccgcggccca agccga 156

<210> 15
<211> 300
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 15
gttcacagcc cataaggtgg gggtggcccg aacctgaaac ggagcctgag ccaggatcct 60

gcaaccaaag tctgaagcgc cccccggtgg gggccgagag cgctgcaggc aggtggnggc 120

gcggggcagg cgggcgggcg aagggagctc cggntacgca ganaacgcgg agcgcccccc 180

tcccacctgc gcgagggcat cctgcccggg ggaggaaagg cgggagtccg aggcgggtcg 240

gattcccagc cagctccctc ctcacaggag gcggcccatt atccggcgtc gcaaagccga 300

<210> 16
<211> 196
<212> DNA
<213> Homo sapiens

<400> 16
ggcgcccagc aggggagcga gggaggaggg tgcagaaaga ggctccgaaa ttgggggaaa 60

ctgacccgtg cttctctacc ttcggaggtg ggacagttgc acgaagtgct agttagaccg 120

gatcagttgg aactgacgga ggactgcaaa gaagaaacta aaatagacgt cgaaagcctg 180

tcctcggcgt cgcaaa 196

<210> 17
<211> 299
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 17
acaccaggag aggggaagaa nccagcacct accgacaggg gtggagctgg gtcaagaatg 60

gtgtggtccc tgctttgggg gaatgctggg gaggtagaaa gccccttcta acggggcgtc 120

actgcaatta ctgcttcctc tttcccataa aactcccccc agtgtatcag aacccccaag 180

gagtttcagt aagcggttct tctgttgtct ccggctgaga ctccagggga acctcaagct 240

cacatggccc tggccgggcc cctgggcagg agcaggcgag aggtctgcgc ggccgctaa 299

<210> 18
<211> 363
<212> DNA
<213> Homo sapiens

<400> 18
gggtatgtgt tacacatccg agataactac acaggcatcg accctgtcca cccggggatg 60

ctagaggggc tgcgctggtt ttactccagg ccatggtgag agccaccgtg aacacagggc 120

tctctcctct gagctgcaga agctctgtgc cctgtcccct gccacaagtc acagactttc 180

ttcatgtgtt ttacctcatg ttaatgaagg agatcttctc caggggcttg atctagtggg 240

aaacagagga ggggggggatt ttaaatttca gtccgtccaa ccctgtagat ctgctgtcct 300

acagtaacgt aaaggatcac caggtaaaac gctgcttctc ccggacgccg ccccgcaagc 360

cga 363

<210> 19
<211> 322

<212> DNA
<213> Homo sapiens

<400> 19
ccggcccgtc cctcttaata tggcctcagt tccgaaaacc acagaataga accgcggtcc 60

tattccatta ttcctagctg aggtatccag gcggctcgga cctgctttga acactctaat 120

tttttcaaag taaacgcttc gggctgcagg acactcagct aagagcatca ggggggcgcc 180

aagaggcaag gggcggggat gggtggtggc tcgcctcgtg gcagaccgcc cgcccgctcc 240

caagatccaa ctacgagctt tttaactgca gcaactttaa tatacgctat tggagctgga 300

attaccgcgg ccgctaagcc ga 322

<210> 20
<211> 255
<212> DNA
<213> Homo sapiens

<400> 20
taataagata ccaaatcggg cgagaaacga aaagctcctg gcctccgtat ttggggccag 60

agacaccgca gggagtcagg tccccgccga caaatcggaa gaggcctgcg ggagttagcc 120

agataatgct ctccctgtcc tacccgtccc caccaatttg ccttttacct gccgcagagc 180

ttgcttgaac caaaggggtt tgcggtcttc tcctcctcaa cttgcgatcc ccaggccttc 240

gcgtcccgaa gccga 255

<210> 21
<211> 406
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 21
atgtgnnaag gctcgctntc catttctctt ttcctccttc tccctctctc atgtgcggtc 60

tccctcaaca tccaaaccaa ccgagtgcgt ctgaggtgaa atcgtgccag acttagagac 120

ggctgccagg tttctctcaa gtcttggctt aacaaaagaa agcaaattac aaaaatggaa 180

attttcaaac tagcgttcag tggtattcaa atcgacgttt gggtagcgca caggcacaga 240

ccgcattcgt gctattttgt gattaaaatg ataccaaaaa tacctccttg ctttggtttt 300

cgtcttcgaa aacgacttct ttccttcttc taatttcccc cttacttttg ggagcggcaa 360

acccctgacc actctagaat tgctaacatt tggaccggcg tcgcaa 406

<210> 22
<211> 210
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 22
gcacgttcgn gcnncgtgta ccatnagctg ccaactggan gcaccnnggn aagggtgggg 60

gcctcctgga gacttngggg agagggatag ccggntaaag ctcctgtcct ttctataggc 120

ataagcgggt ggtcaccacg gattggggat cccgaatccc tggctccaga tagacttaat 180

gaagaagcac ctggatccgg gccgcgncaa 210

<210> 23
<211> 310
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 23
tcacgcttnc naaggctctg aatcctgagg gncagatctc caagaaggag ggaggctggt 60

cctagttccc gaggtcctnn actaggtcta gatcactggg taaaagaagg ggagcggcan 120

cacgtatggg gtaggcgctc tcactactca catctcgaga cctttgccgg cgtagggctg 180

tccgggggga acgacccgcc ttttccggta tcggttgtca tggcggcgcc cagcccagcc 240

tggttttttc cggtagccaa ttgaactaac aaccccgttc cctttaggac taatctgtca 300

cgtcggcgca 310

<210> 24
<211> 304
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 24
ctctggtctg tgntggatac gcgtgttctt ctgcggagtt aaagggtcgg ggacgggggt 60

tctggactta ccanagcaat tccagccggt gggcgtttgg cagtcactta aggaggtagg 120

gaaagcagcg agcttcaccg ggcgggctac gatgagtagc atgacgggca gcagcagcag 180

ccagcaaaag ccctcgcaaa gtgtccagct gctgcactgc cgcggggact cccacagcac 240

catgactagt tcgtgcgact ctgcancanc aaacggcttc cgaggaacac angatcgcgg 300

gggca 304

<210> 25
<211> 379
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 25
aaaacncatn tgnagagcnc ntcggcagag ncgcagctgg ctgacccagg agaaggcgcg 60

ctgggtgtgg ctgggacggc caaggccgcg gcttcccgcg tggggatgcg ctntggcgca 120

aagctggtcc cggcggggcc aggcgtttgt gggcgggtga cggggatcta gggcttccgc 180

tcgngattcc tcttgggctg tctttncggg tttggactcg cctgccaggc tgtgtgcagg 240

gttcccgctg cctctggccg gcaggcgtcc gggctgcagg tgggccggca ggcaggtgtt 300

agcgggaagg gagcacaggt agcgaggtgg gatcggcgac ctggctaggg tgtcggcaga 360

atggaatgcg cggccgcta 379

<210> 26
<211> 625
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 26
gggacgcnag ccagggantt tgatccgttt tgaatgaaaa gaaagagaan ccaaaccaaa 60

cctntcagtc atccaaaacc ttcaggcttc cagggaggtt ttgctataat tttctctaag 120

catgactgtt tctgggggag gggaaagggg tggttgtatt tactgaaaat tcaaatcgaa 180

ataataaatg gccaaatttg gacacttacg gacccaaaca gttttgctca cgccagagaa 240

accgagagca cagggcttgc gtgaagccta tctcggcaga aggcaacatt ctaataaagc 300

ccgtgggaaa acagattaca ttttcgccat gaataagtca tgcagtgaaa aatattgcct 360

acagcctgtc gacttatatt attatcacgt ttttcaactc ggcgtgagga gggagaggag 420

tgttcatatt tgactaggaa ttgcaggatc gatgcaaact ccagggcagc agccagactg 480

gcatatgtgg ggctctccgg ttactttctc tgtatgtcgc gggtgagagg aacagcgagg 540

acaatttagc gcaaacacac gaagggtcgg atctcaaggg ggcagcgctg ggagaaaggt 600

tagggctgna gagcgnanag ncaaa 625

<210> 27
<211> 499
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 27
gnctccncgt tcccctcggg cggaacggag gcaactttcc ggagtctatt tttgttaaga 60

caatcaactc caataactga gctgaagttt ttgtttaaaa agaaaaaaat ctgataagtg 120

atgattttac ctacttgtgg acactagatt tcaattagga aggttttttt aaacggcttt 180

ttgtaacttc gctgcaggaa gcaggtttgt ttctttttct tttcttttta agagaaggtg 240

tatttcactg gtgcaatggc ttggcacctc cggggcctgg gaggacctca gacctcccca 300

gccctgggtt tctccgtctt caagaccaac taggaagggt caagcgggga gagggagtgg 360

agggtcaggt gagatctcag agctgccccg gccggccccc gtctctttct acctcctctt 420

ccagagaacc agcggctcac acccttctca acgcaggaca tgctcggcgg ccaaagccga 480

attctgcaga tatccatca 499

<210> 28
<211> 561
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 28
gggcgattgt tattcaaacn ngntanctct ctgcggggnn gagnaatgng ggcctcgcac 60

ggctncatcc ccgtcgagcn cagggcctcc ctgttctnct agacatncca aggagccaac 120

tcctccgcag aagttccccg cttctgctct tatttccaag cttcgcgctt tctacaaact 180

ccctgttgcc ttgactttga tttccagccg tggtgagggt cagagtgaac cccggcgcgc 240

tccccgacgg catccccgca caccaggata ggagaaattg gagggcctgg gcctcggctc 300

ccgcagtcgt cggaggaaga acccaccgcg gggtccccaa gggaaagtga agaggcccgg 360

gatttttcca aagcgctgcc aggaccccga aggaagggga ggagtcacct gaagccgggg 420

aagctccttg ggtgctctcc ttggatcctt atgttcactg actttcgcga ngccccctgg 480

aggnggaaaa tccgcgctgt ttcccccaac ttaacttcac gcggccgcta agccgaattc 540

tgcngaaatc attacactng c 561

<210> 29
<211> 717
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 29
actctccgcg gtntcntggt gcctcacagg aggtggggct ccctccaccc ggtccccagg 60

cctctccctc tgcccgagct tcccggtcct gcctccttcg cctcgcctgc ctgcccgact 120

ctgaaccctg ctcctcttct aactaaaagt cagtgtttta tttcctccgc agtccaatgc 180

ccgcgtttta ccttattcaa taagaagggc ttcatttatg gcaagacagg acagccaggt 240

aataagggcc tctgcacacg cgggcccatt ggaggggcgg aactgcgaag tcttcccgga 300

agagcttcct ggagagaagg ggaacgagcc agcgtttatt gagcatctat tatactaagc 360

atctgcttgg cagttcacga cggtcgcatt ttttcatcct tacagcgatc cctattgtgt 420

cgcttgcttt aaagcctcac agctcacaaa gggctgggat ttattccaga tctctctctc 480

agatgccatc tcacttccag gtgtctctgc tgctttgaac gcgggaaacc cacgcaaagg 540

agtgatttcc aaggccttct gtttggaata tctttaatcc tccccttatt aactggaaaa 600

actcccacgc atccttcagg gctcagctca aatgtccttt atntctgcag ngaaactttc 660

ccaaggaaaa ttagttacac agctaatttt agataaattg agccagttga tagaatt 717

<210> 30
<211> 280
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 30
tgatggatat ctgcagaatt cgggctttgn gacgccgggc acgcagtagg gaaaacagta 60

ttaaaacgcc ctacagaaaa tctcggcgaa gtccccggag aactctggtt tctaagatca 120

gctgggcgca ctttctccgg gacgtccctt cttctcggtc tcagcgcctt cctgccctca 180

gccgcgccng tnttgttttg gtggcaaact gaaataagaa atggaaatat attggccttt 240

gctgctgcca gggatgagag gttgttgacg tcggcgcaaa 280

<210> 31
<211> 270
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 31
gnggnngnna nncggcgatg gatntnngna ganttnggtg atggatatct gcagaattcg 60

gcttagcggc cgcgaacaaa gagcgaacca aaggatgctt cgaattttta aaacggaatc 120

tctgcaccca aatgcaggac tggtgactta aggagctgcg aagtctgatt taccgggcct 180

actctcgacc tgccccccac ccccagctca gggggacctt tttatcntga acgccagagc 240

tacnnaccaa gtcgggtggc cacnnccaaa 270

<210> 32
<211> 347
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 32
tttggannta ngggggcgtg gcgtggatcc agtttccccc ggccaggtcn gcttcccggt 60

ctcaaccatt tcgcgctctg ctctgtccgc tggtttgtcc ctgcccggtt cctctccccg 120

ggcctgtcag cctccgcttc tctggaggtt cctgggactc atctctgatc caccgtcttg 180

cgttctctgg gcgcatcgac ttctctccat cttcgggctc actcctgact ccctcgctgc 240

cgccccgggg gtttccacgc gtgtctctaa ccgcggccgc taagccgaat tctgcagata 300

tccatcacng aantctgcag anatncatcg ncgaannnca ccgcact 347

<210> 33
<211> 342
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 33
gtagggcgcc gccgtgacag attagtccta aagggaacgg ggttgttagt tcaattggct 60

accggaaaaa accaggctgg gctgggcgcc cgccatgaca accgataccg gaaaaggcgg 120

gtcgttcccc ccggacagcc ctacgccggc aaaggtctcg agatgtgagt agtgagagcg 180

cctaccccat acngtcggcc ggctcccctt cttttaccca gtgatctaga cctagtctag 240

gacctcggga actaggacca gcctccctcc ttcttggaga tctgaccctc aggattcann 300

nnctttgctc acgagctcca acccnacnca tccaaannnc aa 342

<210> 34
<211> 370
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 34
cattgtttac tttcgtctaa acgcggtgga agcccatgga agaaagcggt tagcagcaag 60

gcagagccct gctccctctg cagccccagc tcccagcgcc ctgggctttc caggcacctg 120

tccgggtagg ggattgaggg ccgtggccag gcccgcactt tcctgctagc cgcagctggc 180

cacatgccca tctgaccctc cgagttctcc tctaaaaatg gggctgacag ccgctacctc 240

acaaagtcca caccgggctc aacccgntgc cttcctcccc aacaggactc tgccaccctc 300

cctcaggatg cctgagggcc ccganctgca cctggccagc cantttgtga atgaggcctg 360

nggggcgntt 370

<210> 35
<211> 213
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 35
aaaatacnan taaagcgatg cttcgaattt ttaaaacgga atctctgcac ccaaatgcag 60

gactggtgac ttaaggagct gcgaagtctg atttaccggc ctactctcga cctgcccccc 120

acccccagct caggggacct tttgtctgaa cgccagagct actgaccagg tcgggggggcc 180

gcggcccaag ccgaattctg cagatatcca tca 213

<210> 36
<211> 173
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 36
gacnncgggt ttgtgtgtaa cagggtcagt ccccgtatct actttgcgaa agcttcgagg 60

cgagcgtgaa gtcaagggct gcggtggatg ggggtaaaan gcctcctcnt cccactgcct 120

gcnccgtctt ggggtaaccc ctanccccca cccggngttn cnctttaatg ctc 173

<210> 37
<211> 369
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 37
tcactgtgcc gggtctctcc tncccggtcc aactccctta cttgtcctca tctctgtccc 60

caaggtccgt gacccgcgga ggtgatgggg gggataggag agccccaggg accgcagagg 120

tgacacaatc gcccgcccgt cctccctcgc tgggagccga ttcagcctgt gccgagcctc 180

tcccttcgcg tgcctctgcg cacagcggtg gcaccgcagg actccgggtc ccccccggct 240

ctccatcggg aagccggcaa atgcgcttcc tcagccagac cgcggcgggg tgggggcggg 300

gggggcggaa gttgaaatac tgggacagaa acacctgccc gtcccaaggg acggaaaact 360

ggatgccaa 369

<210> 38
<211> 123
<212> DNA

<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 38
gtcccttcgc cccgcttttn ctttccccna ggtcccagcg nccgaaccgg cggatgtcca 60

cgaaacatag ggcgagccgg gggccangcg gggccgtgta aaatctcntg tggtcatttt 120

gtg 123

<210> 39
<211> 450
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 39
ctagccctgg aagagaatcc gaggctcagc cntgctgcag cacccaggac actgcatccc 60

agcacctgcc cgaagatcag cccaggaccc aaaggaaagc aggctccaag ctccccggaa 120

gccaaggaaa ataggaaaac atatcctgcc ccggggacac cttctggaac tatgaccaca 180

tgcacttgac cttccggaac aatcaccgca tgcacctgac ctcccggaac tgtcaccacc 240

gcgcgcacct gacctcccgg cactgtcacg accgcgcgca cctgacctcc cggcactgtc 300

atcaccgcgc gcacctcacc tcccggaact gtcaccaccg cgcgcacctg acctcccggc 360

actgtcacga ccgcgcgcac ctgacctccc ggaactgtca tcaccaggcg cacctgaccc 420

cccggcactg tcacgaccgc gcgcacctca 450

<210> 40
<211> 593
<212> DNA
<213> Homo sapiens

<400> 40
ggaccaagct gggtaaactg ccgacagctc cattgggcag catgtccacc cctgatgacc 60

aaatcccacc aaacgtgcag ctggcactcg gccgcctttg tttccttccc ctagaataaa 120

actccgctgc tttcccacgt tcctggagca gcagccggaa taaagcgccc atggccttgc 180

cctttgagtc tcggaggatg tttgccactc caacaatgga cttttaaata attcaggggt 240

caaaaggcgt gtgtgtgggg ggggagaaaa gttacaaatc agcacttgaa accgaacaca 300

aacaaaaatc aaacaaatcc gaactaatat aacaaatcaa aactttgatc tttagaagaa 360

aacttcaacc ttaatgcttc caggaggaaa gcagaaagga taatgactga attgtgaaaa 420

cgagccaaaa tgttccacca ctgatgtcac acacacctat gactccctgc acagatccac 480

ggtcccgggc gctgaatccc cgcaaccctc tgcgcccaca gaggttaaac tctcgctgct 540

ggcgacttcc gcttcctggc ctaaatctga cacgcacgac tccccccgcg gca 593

<210> 41
<211> 457
<212> DNA
<213> Homo sapiens

<400> 41
accaccaacc aaatagggcc tttcctgtta acgaccacgc ggcaaggggg ccgggccctc 60

gcacgcctcg acggcctccc ccactccaaa gggactccga tttcgcagga tctcccgcct 120

cccgcctctg ctcccaacac cctacgtttt tctcttcctc ctcatttacg tatttacaat 180

aaaacagcga agctgcacag tctgtctcta aatcaaacgc ggttaccatc aaagcctcag 240

actctatgtc tcaaccgcaa aaggtctgac aggaaatcaa ctcgggagtt tgtcaattct 300

ttaaactcaa agctctgtta acgaaatctg gatctttcct cgctccccac ctgcctcccc 360

tgacaggaga atgactgtaa aaggatcctg tcgtccccga aagtcagcac caagcacttc 420

acaaattgtc aaatctcaaa agcttacacg cgcggca 457

<210> 42
<211> 211
<212> DNA
<213> Homo sapiens

<400> 42
gcctgacctg aatgacgcgc atgttgaggc cggtctcctg cgccagctgc tcgcggatgt 60

ggcgggtggg cttgggtgta gcagcgaagg cggccttcag cgtctccagc tgcttggctt 120

tgatggtggt gcgcggtccc cgccgcttgg cgcccaggtt ctggtcgtca ttctcgttgc 180

tacccgcttc cttgtccgac acgtcggcgc a 211

<210> 43
<211> 141
<212> DNA
<213> Homo sapiens

<400> 43
aaatcatctc cgggggccca gcacggacac gctccagacc cgtgagttcc ccagcgccgt 60

gccgggaggt caggggcgct gaaagaagga agaattcagc cacctctcag catccctgtt 120

acctcgagga cgcgcctctc a 141

<210> 44
<211> 559
<212> DNA
<213> Homo sapiens

<400> 44
acccactttc cattaacact aaataaaacg catccatgga tttcctctcc attccgaggc 60

aacaggagtg catggcacat tgccctactc ccctgaagct cttcgctaac ctaagactcc 120

agggtgagga agttagctgg agctttttaa agtgcatctc caaagagaat tttgctcaca 180

ccatgagagc ccccaagaaa caccagggcc cccttagatg ccggagacca cgccctccag 240

gaataagccg caccctctgc ccagcagatc cttgcgcgag tagccctctt tccctggggc 300

taatcaagtg catgccacat gtcaccactc tcagctggca attcttcctc agaggcgcag 360

actttcacgg aatccccagc agggggggtt aagagattca ggggaggccc cgcccgtgcc 420

ttccacaaaa gtcgctttac cgtggctcgt gtcctgcggc cccaaggggg tagcctggga 480

cgtgtattgg gagggcatag aggctccttc caggacaagc tgccagcctc cagtgggcaa 540

ccatgtgaga ggcaaaatt 559

<210> 45
<211> 433
<212> DNA
<213> Homo sapiens

<400> 45
gcgaacagca caaaggcttc attcctacga gagattaagt tttagagcaa atggacacga 60

tcgttaaaga atttgatatt tccatgtaaa ctgcattagc aggttatgcg atccaaactc 120

acaggaacaa ctccaactct cggccatgcc ctatttcatg tctagatttg tttaaccgac 180

ttacatcata atccaagaat acgaactaca gtatattctt acagcaaagt tattccttaa 240

aagcaaaacc gagccacctt tgaaaacacg cacacacatt atccacggca ctaaaacccc 300

agtcttgacc gagaaagacc aacaacttgg gggggaagaa aacaacttca gagccagagc 360

tcccaaagca gaaagcgctg gcggctgaag ggcacacgag gttccgctcc cgggcgaacg 420

ggcggcgtcg caa 433

<210> 46
<211> 487
<212> DNA
<213> Homo sapiens

<400> 46
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cctactgtgg accacgactc tcccagcagc gggcgtttaa tatccagtta gcaggttctc 120

accaccccct cgctggctcg aatacagcat ctgcaccgag ttcccgagaa tcgtcaaccc 180

agcaaatccc ttaattggtg gacatgaaaa tccagggctt tgtgctgtaa taacagagtc 240

ctgggggcct ggggagtttg tgccgcttgg agctcaggtt tctgggacag aggctgagcg 300

cagggcaggg aggcaggtct cacctggcac ctcccagagt cctcgccgag cagatggaag 360

cagaggctct cgcgcccggc ccccgccggg agacctctct ctctttccct cggcctgctc 420

tgccctctcc cgccttctcc ctgtctgatc cttctctgct gtcatgttct ttgtcctcgc 480

gccccga 487

<210> 47
<211> 403
<212> DNA
<213> Homo sapiens

<400> 47
gtcatataag cacaaccatt cccagggcca ccctggatgc atcagatcag tccccccact 60

ggtgaccaca atggctggct cagagtgcct ttgaacagac aggagaaaca gacttcttgg 120

agggagggac cttcccacag ggaatggcca aggagctagg tcttcagggc ttgcatggcg 180

tggagtgtgt gctcaggtgc acagtgaagc aaacctgagg ggacttgggc cctgcgtcct 240

ccagcacaca cgcacccttt cgccgtcaca tccggggcac ccacccgtgg aatatgtgag 300

ccgcacttgg ccagccacga gttccagggc caggaagtcg tgcttctcgt tcaggcgccc 360

gttgtagaag agcagcccgc tctgctgcac tgtcgcgtcc cga 403

<210> 48
<211> 155
<212> DNA
<213> Homo sapiens

<400> 48
ggcgtggaga ggaggggggca gaaactcagc cgcccctacg tttgctaaac tgcgtccgcc 60

aggggggcgta tttttctaaa acgcacaaga cgtttcgtgg gttatcgatg gtctcttgag 120

cctccttgac tgatggggat tgaccgggcg ggata 155

<210> 49
<211> 256
<212> DNA
<213> Homo sapiens

<400> 49
tctactgagc ttttctttaa gtggaaccag aagtgctggg atgagaggga aaggatggga 60

gtgcgtccaa aggtggacag caggtcccca tccctggtgg gagtgagact ggacggcatc 120

ccccggaaag gtggtttggg ccttggacaa ggctagaggc aggagtccat gatgcagaga 180

tgacacagtg cccctccgcg tgtgagtcca cgaaggtcac tactgaggct ttgtgcttgt 240

aaaaggccgc cccgca 256

<210> 50
<211> 224
<212> DNA
<213> Homo sapiens

<400> 50
tgcggggtcg tgggggaacc ggcgggagct gttcgctggc cggcctcact ggagtaggaa 60

ttttagatga aactgagtcc gtttctcctt gaaggcaggc agtattctta gatctactat 120

tcatttaaaa agaaggaaaa gaaaaaaaaa tgactgctac ttactgagaa gaaaatttct 180

gttctcctcc gattccgctg atcccgcttt atccgcgcac ctca 224

<210> 51
<211> 313
<212> DNA
<213> Homo sapiens

<400> 51
gtggctggga cggcccaggc cgcggcttcc cgcgtgggga tgcgctgtgg cgcagagctg 60

gtcccggcgg ggccaggcgt ttgtgggcgg gtgacgggga tctagggctt ccgctcgtga 120

ttcctcttgg gctgtctttc cgggtttgga ctcgcctgcc cggctgtgtg cagggttccc 180

gctgcctctg gccggcaggc gtccgggctg caggtgggcc ggcaggcagg tgttagcggg 240

aagggagcac aggtagcgag gtgggatcgg cgacctggct agggtgtcgg cagaatggaa 300

tgcgcggccg cta 313

<210> 52
<211> 385
<212> DNA
<213> Homo sapiens

<400> 52
tacgttgcgc attcattctg ccgacaccct agccggtcgc cgatgccacc tcgctacctg 60

tgctcccttc ccgctaacac ctgcctgccg gcccacctgc agcccggacg cctgccggcc 120

agaggcagcg ggaaccctgc acacagccgg gcaggcgagt ccaaacccgg aaagacagcc 180

caagaggaat cacgagcgga agccctagat ccccgtcacc cgcccacaaa cgcctggccc 240

cgccgggacc agctctgcgc cacagcgcat ccccacgcgg gaagccgcgg cctgggccgt 300

cccagccaca cccagcgcgc cttctccagg gtcagccagc tgcggctctg ccgaagcgct 360

cctccgctcc tttctcgcgc cccga 385

<210> 53

<211> 307
<212> DNA
<213> Homo sapiens

<400> 53
aacccggctc ggttcggcaa ggttcaggga gacaaggtag agaaggctgg ggtgagcaag 60

aagtcgggcg gccgatcgtc agggccacga gcctcgcctt gccttcttgg aatcccaccc 120

aactttaaag gcccaaagat cctgaaaatt ccgaaagcga aactgcgggc tggtctccag 180

aagtttgaga acggtctccc aggctttcca gcgtcgtccc gggattctcg gacaccacaa 240

acgccatcaa ccacgagcac cggtgtccgt ggctattgcc ccgaatggtc cccatccgcg 300

tccccta 307

<210> 54
<211> 182
<212> DNA
<213> Homo sapiens

<400> 54
cgatgtcgaa gccgtttgga gggaacagcg gtttccaagt tcctgctgac ttgagaagcc 60

tctgcgggtt tccgaatctc cggcgcactc ctgggcgcgc tgcgggagct gtagctcagc 120

cagccaggga gtagcggctt tcatccgccg ggaggagtct ttcgagttca atcgcggggg 180

ca 182

<210> 55
<211> 523
<212> DNA
<213> Homo sapiens

<400> 55
tcgggtttga tccgccccaa ccaaataggg cctttcctgt taacgaccac gcggcaaggg 60

ggccgggccc tcgcacgcct cgacggcctc ccccactcca aagggactcc gatttcgcag 120

gatctcccgc ctcccgcctc tgctcccaac accctacgtt tttctcttcc tcctcattta 180

cgtatttaca ataaaacagc gaagctgcac agtctgtctc taaatcaaac gcggttacca 240

tcaaagcctc agactctatg tctcaaccgc aaaaggtctg acaggaaatc aactcgggag 300

tttgtcaatt ctttaaactc aaagctctgt taacgaaatc tggatccttc ctcgctcccc 360

acctgcctcc cctgacagga gaatgactgt aaaaggatcc tgtcgtcccc gaaagtcagc 420

accaagcact tcacaaattg tcaaatctca aaagcttaca cgcgcgggca ctccggaaag 480

gctgtgggga ccacccaaag cacccccctc cacaccgcgg gca 523

<210> 56
<211> 795
<212> DNA

<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 56
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tagccctgcc tgcctttcta gagggcttct tggatttgca gcttctaaca agttgctctc 120

gccacggaga agctgttatt atgacaaaat atttggggca ttatcaaaat cacacaggct 180

gctgggctgc tgtcggtttc tcgccagggc cagtaagcag ttacatttgg agttgctacg 240

tgttgtttgg gggccgggct gtggagagtg actgagccag tatttttcat ccaaaattct 300

gcaaattgaa ttaaccacaa ttctagtctc acctcccgtc tttaaaaaaa taagttgaag 360

aaaaggtaaa tattagagat aaggcagcat ctagtgactg cggagaggca caagctggtg 420

ggcgagggtt gggggagtca gcaaagccct tcaaaacctc cccgtttaat tttctggctg 480

tctctgcatc ctgttgccag aattccaaat gcttggagtc atttanaggt gcgagaactc 540

aaacgtcgtt ccacttggaa aggggaccgt ttaacgttaa attccattag cacctaaatt 600

gtttcttaaa gacatccgct cagacacagg actcgaaagc gagcatttca tgcaaataaa 660

tttctcaaat tttaaacctt gttaaaagct tgtctcgcac ctcggctccc tccccttccc 720

cggaaganaa caataggccg ntggcgcatc cccacttcgg antaaatatt gacgggggaa 780

gttgctaaaa acatc 795

<210> 57
<211> 438
<212> DNA
<213> Homo sapiens

<400> 57
gcctgtgtgt aggggactgg aggtggggga acctgttctt ttcttgtgtc tgatcctggg 60

gctcgcttcc tgggtcctag aacagcagcc aggacggaag aaactgttca cgttgcaccc 120

ctttctctaa gattcccagg ccaagagtag ctgcagaagg tggccctgaa tctatggcct 180

ccttctctct gcctgacccg gctagtggat ccggagaggg gaccagggag agctcctccg 240

agcaggggtc cttcgggaga cagagagggg tccaggctga gagaactctt caagcatggc 300

gagtctgcgt tatagaatcg ggcgggcggc tcaacttggg ggaagcacca agaagagctg 360

ggcgacctgg agcgcagaac cggctttggg gagccacccg gcggggcagg ggtagcacgg 420

agcccgggcc gcggccca 438

<210> 58
<211> 611
<212> DNA
<213> Homo sapiens

<400> 58
gcttcccct tcctttctcc cgcgctgccc ccttgagatc cgacccttcg tgtgtttgcg 60

ctaaattgtc ctcgctgttc ctctcacccg cgacatacag agaaagtaac cggagagccc 120

tacatatgcc agtctggctg ctgccctgga gtttgcatcg atcctgcaat tcctagtcaa 180

atatgaacac tcctctccct cctcacgccg agttgaaaaa cgtgataata atataagtcg 240

acaggctgta ggcaatattt ttcactgcat gacttattca tggcgaaaat gtaaactgtt 300

ttcccacggg ctttattaga atgttgcctt ctgccgagat aggcttcacg caagccctgt 360

gctctcagtt tctctggcgt gagcaaaact gtttgggtcc ataagtgtcc acatttggcc 420

atttattatt tcgatttgaa ttttcagtaa atacaaccac ccctttcccc tcccccagaa 480

acagtcatgc ttagagaaaa ttatagcaaa acctccctgg aagcctgaag gttttggatg 540

actgagaggt ttggtttggt ttctctttct tttcattcaa aacggatcaa actccctggc 600

tcgcgtcccc a 611

<210> 59
<211> 291
<212> DNA
<213> Homo sapiens

<400> 59
gagtttggca ggccccggat tccacaaagg agtaggcgcg gccagccgcc tccagccctg 60

agctcagtaa attcggtgtc ctgaatgctc ccttcctgtc cttaccactg cgagctctct 120

tgggacagct ttctaggttc cactgcgacc tactttccgc tccctgagtg cttctttgct 180

gaaactgcag gcgaaaagat ctctttccca gaccgcagcg cactttgaga aggggctcaa 240

agtcgcccgc tctgaatccg gcaccggcaa ataggagtag ccgcatgcgc a 291

<210> 60
<211> 226
<212> DNA
<213> Homo sapiens

<400> 60
gaaaacagat aaaacgccct acagaaaatc tcggcgaagt cccggaggac tctggtttct 60

aagatcagct gggcgcactt tctccgggac gtcccttctt ctcggtctca gcgccttcct 120

gccctcagcc gcgcgcagct ttgttttggt gacaaactga aataagaaat ggaaatatat 180

tggcctttgc tgctgccagg gatgagaggt tgttgacgtc ggcgca 226

<210> 61
<211> 580
<212> DNA
<213> Homo sapiens

<400> 61
ctgtgatgca ctcggcggat ctcggtggca gctgcctcct tcatctccag tgacgcctgc 60

atgctgtcct aggcagtgtg aggagtgaag atgagatttg gcgcatcttt caacggagtc 120

tgagcaaagc taaagggctc cgattcgtgc aagccaaggg ctgcccctcc tatcctgtcc 180

tccttgagga cctgtgctaa ggctttctca tccaccaggc caccatgggc tgcgttcaca 240

aggaatgctc cctgtctcat ctgctttata gtaaagtcat tgacgaggtg gtggttatgt 300

tcattgagat tgctgtgcaa cgagacacag tcactctgat acagcaaacc ctgcagggtg 360

tatcagggtc ccctctgcat gccctgggac ctctctatct tgtcctacaa gtaggggtca 420

taaaatacga cgctgaatcc aaaggccttg gctcaaactg caaccgcctg cctcatgcaa 480

ccgaagccca tgaggcctag cgtcttccac gaatgagggc cactcccatg gccacctcga 540

gaatctgctc cacgctctga acccgcgcac ctcaagccga 580

<210> 62
<211> 633
<212> DNA
<213> Homo sapiens

<400> 62
gcccaggaga agccctccac ggtgggcgtc ctcctagaca accagcaccc cctgcaggca 60

ccctcgtctg gcagaatcag ccctttccca cctgcaggcc cttctcagcg cctctgactt 120

cccacacaca gcacaggtta caaactggtc cctggcagtg cactctagcg ggcctctctc 180

acaagttctg cgggcctcgt ttcatggaaa gcgggttgtg gattcctgct gcccttggat 240

ggcccctgcg cacgcacacc tctgagcggg cactgagcga gcgtggggag ctgctccctg 300

ggaactaggc aggagctttt aaacaccctt acacacagcc attctgcggg aatacatgct 360

ttcccggtaa ggcttttact gttcattcca ggtaaattgg aagtcgcaca ccccaagctc 420

caaatacaac tcgttagctg gcaggtctct gaagccaatt ccttctgagg aaaatggaga 480

taatagcagc taccctccca ggtgactggg ggagaataaa gtggctgtgc atagtggtgt 540

ttgcagctgg tggctgctat tatccttcat tacagcttgt aaaaagggtg tctaggccat 600

ttacacacag ataggccggg tggggtaagc cga 633

<210> 63
<211> 703
<212> DNA
<213> Homo sapiens

<400> 63
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gaggagagaa ccaaaaaact tggatgtttc cgtaaactag attcgtcaat cctcgataat 120

tgaaagtagt tccagtatgt cagccaccgg ggttccctgg ggagctaacc agtcctgaag 180

gaagtatgaa gaggaagagg aggtcttcag ttaaggggat gaatttgtgc agtcctaagc 240

cctgcaaagg tgctggaggg aggaagaagg gcaggaaata aaagatggaa gaaaatttgt 300

tttttatcca cttagagttt tatctttaat gatgggaaac agtgctgctc tcaggaaact 360

cagtgtggag atctaggagt tcacggttca tagtccatta ggagcaggaa aaggatagag 420

gacatttata aagtaacatc caagtccaaa gtaaaatggt ataaattgtt tcccatgata 480

aaggctggct gagtaggtca ggaaaggtct tgtcagacca tatgtgctgt ttcaggctgc 540

ttcaaattct tttaggacag tggtggatat gagtgaagac ggggcaggca ggccacatct 600

cttagaagag gaaggtgatt gccacgtctc cttcctccat gctgatggca aggcgtgcgg 660

gctgtgttct cttgcagcca gcgtcccatg ctcggcggcc aaa 703

<210> 64
<211> 420
<212> DNA
<213> Homo sapiens

<400> 64
gtgacgtgcg gaatacacgt gatgtcgggg acaggagcgg gctgaagagg gcacgatccc 60

acgcggaggc caccctcac ccggggtagg agcccgctgc acttgctgtc gctcagcccg 120

ggcgctgcac cacggcagcc gccatgctgc ttaaagccgg tcatgtgacg cgggagccag 180

ggtggaaggg gtccccgcgg gcaagccttc gacacgtgac ctgccacccg actacggaag 240

cctcttgggc gttccgcccg ggctcacatg tcatgtgacg gccggccggt cgccggagta 300

accaggaact ttcccagacc ctgcggtccc tggagcgtca aaaagagcgt ccccgtgact 360

aggtggagtc gcctgccctt ccgaatctca gctgtcttat ctggaacccc cacgcggcaa 420

<210> 65
<211> 496
<212> DNA
<213> Homo sapiens

<400> 65
gcgctgcacc aatttagagg gtagaaaaag gagttagaag caaagaggaa aaaataaata 60

aacaggcaac aaaaacccaa cccagccagc ctgagccatt tgcattagtg ttcatttagg 120

aaattagcag acgggaaacg ctggggagtg gagtgggccc cggccttggg gactgcagag 180

cccgctcagc cctgggtggc tgggcccaca tggctgtcgc caggagcaca ggaggaccca 240

gaggtggccg agggagcctc gccgggctcc ggtatgggtc ctggcccctc acaggtgcga 300

gcctggccca gtgactgtgg acgctgtggg agagcaggcc tccgatacgc agggctggga 360

ctgctgacct ggaaggtggt gccgggcgtg tctggtgaag gcgccgttgg cagctagaga 420

gagacggcgg atggggtgac gccataaccc acggtcccag ttttgaggct tgacggtgac 480

ggaaaaggac gtcggc 496

<210> 66
<211> 637
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 66
cgccgagccg ggatgagcaa ggcttcctgg aggagagggc cggcctgagc ttggaaggat 60

ggggaggagc cactggctac aagggtgtag aggtgagaac cagtgtgacc tgcccatcgc 120

tggtcgtctc tgggtcattc agctgaaatg gcatctctga gctgagagga gtgttgcctg 180

taaggagcta ggcatcagcc cccagtagag gggcggccca ggcacagccc atagccgcag 240

acttagtgag tctagctagg gagacagtag aggggccaaa atgaggacac aggtcaccaa 300

aaatcctggc caggtcctgc cactacctgg ctcagcgacc tgccccccg agcctcagtt 360

tcccccattg gtggaatgga gtgaggaaga cgcgcctccc ggggctgcga tggagaattg 420

agtcagagtc tgggggtgct gggagggctg gggagcagcc tccctgagcc tcagtttccc 480

tggctgggga atgaggacct tgctcgtccc ccctcataag gggaagctgt caggaaagtg 540

ctttcaacgc tgagccattt cccagtggtg cacaattagc tttccagagg attttggtgg 600

attctagagc tngagggctg ggggatnggc ggccaaa 637

<210> 67
<211> 595
<212> DNA
<213> Homo sapiens

<400> 67
gccctgagct cttgagggcc tctgcagttc ttgggacaat tctgggacta tatctttggg 60

ccttggtgag atctagaggc tctaaagtct ttgggagggg tcctgagctc cgtggacggc 120

agggtcttgg gcactcactt gcattcttga ggggtgtgtt tggcctcgtc cgtgcaggtg 180

tagaatttcc cctgtagaga ggatgtctgt caagtaggtt cacccttcat cacactcccg 240

cccagacccc tgcctggcat tccctccagt gtttgcccca ccttgaagag ctgcaccccg 300

atgcaggcga acataaattg cagaagtgtg gtgacaatca tgatgtttcc gatggtccgg 360

atggccacaa atacacactg caccacatgc tgcgggcacc caagcatatg gctactgaac 420

actacaggcc acagtggtca tggggcaggg actctggtca tagatgcagc tgagggactt 480

gggctgggga catgtggtga tgggtcaggg atgtatggtt agcaacatgt gttcaagagg 540

cagtgttatg ggctagagac gtgtgggcat ccaccaggaa taagtgtttg ccggg 595

<210> 68
<211> 580
<212> DNA
<213> Homo sapiens

<400> 68
gagtcaggac ggaggacgcg gcaggtcaca gagcccacca agtccgaagc tggaagttca 60

gattctttga tattcaaagg tggatcatct gtgctttttt ttttttatca gtctctcact 120

ttttatccat catctaattg tgacagctta tttgccttta taccataaga tggggagtag 180

ggttgagatg aaatccaagc atcgtttccc ttccccgatg gtcgcctccc tggggtgaga 240

cgttcgacgt gtcagacttc accaagagca tctcccgcct cggtgcagta atgaacttgg 300

aaacgattta ctccggcact tggttcctgt ctccataaat gcggctgctt taaagggaat 360

gtaaaaaggg ctgtaaattg gtattgattg ccggtggtct tgaagaaccc caactgagga 420

ttgaccgttc cttggagtga aggctccgca ttcagacgcc tttcgcctta cgtcatcata 480

attgagaagg gaaaggagac gtgttagttt cagtctgatt atttaccatc aaggcataaa 540

cacttctcag aggcagcgga acccattaaa ccggcccgta 580

<210> 69
<211> 589
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 69
acacgggggg caacctcttg cacctggctc cctgccctcg gtgccacgtt tccagggttc 60

ctccacgtcg caggctgtgt cagcctcgct ccttccactg cagaattgcg gtccacagcc 120

tggatgggcc actctccatg tatccacctg tccctccgtg gctgctgggc tgagtcgctt 180

ctgatgctaa caagaggcgt ccggctggac taaggccccg gaagctgaga actggagggc 240

aggtgcgggc atcgggcaga gcagctccag caggcaggac ctggggcctc caccctgcac 300

ccctgtgccc cgcgtgtggc ggaaccgccc cgaggggagg ctgtcaccac ggtgacaggc 360

agccccacgc gagcctgaga accctcagcc cacctttttc tgtaatcaca gcaggcatct 420

ctccggcaag tcaatccagt tccagctggt gctgcctccc ttgcctcatg ggctttattt 480

tagaactctg agcaataata aaaaagacgc tacccgctac aatagatgtg gcagagaatc 540

tggctcttca cttcatcana gatcaccctg aaatgatggt tgttgttaa 589

<210> 70
<211> 748
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 70
gctacatctn ctctacattc taactaacac ttgttatttt ctgtttttgt ttgtttgttt 60

ttaatagcca ttctagtagg catgaagtgg tgtttgcctg ctttttttga tggaggtgga 120

ggaatagggt ggaattggtc cttaaccatc aattaagctg ggggccttag acctctgtga 180

attggctgtg acaatagcta aaggaggctg ctacctcata ctgaagagat gtttcctaag 240

tttgtcaccg gagagggcac cgaaccaact tattgtcttg gagggaagaa gcagcaaggc 300

agaagacttg aacttctcag agaaaaaaac agtctacaga cttcatttta tgctgtcctc 360

acacactact gaaagctcta ccctggggac ctggcttgac ttctaaccta cnnctgtgtt 420

atttaggaag agctcccagc tgctctgagt ctcagtctcc caatcagtga aatggaggca 480

atagcacctg cctggctgca tcgccccaca gtgctgcaat gagcatccaa cgagagaaag 540

cttgtcacct gtgttgcaaa ctaagttaca caaatgcagg cagtagcagc tagaagaaaa 600

tggttgggaa tctgaaaaga attaaagccc cccatgaatt tcttctcacg cctcctccaa 660

aagccaggga ctgcttcacc ccgcctccag gactgctcgc tccagcattt ccggcagctg 720

ctgacagaat gtatgttgcg gctgtccc 748

<210> 71
<211> 599
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 71
gatgactgtt gcccgagctg aggccacgac ccaaccccga ggaagggaga acagcttccc 60

atgaagggca tggctgctgc cccataatcc cagggcagga ataaagggga tcttggacta 120

ggcaatcaaa ggacttcctc tccctctaag gccaaggagg aaatgtggct gggactccaa 180

gctctgtgga tgcctggagg tgccagcagc tggggatcag ctggccccac ctgcagagcc 240

agccagtggt ccccctgcat ctccaaggtt gggtctatgg gctccaagaa caggtgtttc 300

tcagggtaac ctcagcccct acaacttcaa ccaagagagt gaaggggagc agccctggag 360

gccaatgagg agggggatta gtggtcactg atgacaaaga catccctgtc cccagagcca 420

gcccccttgtg agcagaagaa tggctgccgg gcaaaaggac ctgctatgcc ctccccatac 480

acatatcatg ncacctgggg accctctgaa taacaggggg cngctttaga gtggcttnat 540

taccaacaag aggcccagaa gggctagagc acacgatttc atgntcggcc gcatgncaa 599

<210> 72
<211> 614
<212> DNA
<213> Homo sapiens

<400> 72
gtgcgctatc acgactgttg cccgagctga ggccagaccc aaccccgagg aagggagaac 60

agcttcccat gaagggcatg gctgctgcca ccataatccc agggcaggaa ataaagggat 120

cttggactag gcaatcaaag gacttcctct ccctctaagg ccaaggagga aatgtggctg 180

ggactccaag ctctgtggat gcctggaggt gccagcagct ggggatcagc tggccccacc 240

tgcagagccc agccagtggc tccccctgca tctccaaggt tgggtctatg ggctccaaga 300

acaggtgttt ctcagggtaa cctcagcccc tacaacttca accaagagag tgaaggggag 360

cagccctgga ggccaatgag gagggggatt agtggtcact gatgacaaag acatccctgt 420

ccccagagcc agccccttgt gagcagaaga atggctgccg gggcaaaagg acctgctatg 480

ccctccccat acacatatca tggcagctgg ggagccctct gaataacagg gggcgcttta 540

gagtggcttc attaccaaca agaggcccag aaggggctag agccacacga tttcatggtc 600

ggccgcatgc gcaa 614

<210> 73
<211> 552
<212> DNA
<213> Homo sapiens

<400> 73
aagcgcccac agatggccaa gcatgtggag gagagcacaa tattttattt aaatatccaa 60

atacgaacac attcccgcat ggcaccaaca gccgcctgaa cacgcccgat gccggcttgt 120

gctttttccg ttttgtctag aaatttgggt tgcactaaat tctcagctga atgaagatga 180

gaaggggctg gcagaggggg tggctccagc tctctgagaa cctggctcct tcccgggtgg 240

cagggagaga tggcccctgg ggagacgggg agggtgcact gcctcatgcc caaaccacca 300

gcttctagtt gagaaatcag aattttctct gcagaataag gaaaaagcat tgtcaccatg 360

attcacgtgg agctggccac actcaggaaa ttcaatgggg tcccacaggg gctccgaggg 420

ggaaggagag ggcctgggac atgcccctcc agccatcatg gaacaggatg ggcagggccg 480

gccctcactg ctctctaaca gtgaaaagcc acatctccac tttggaaaac acaggcatgt 540

gagagcctgg gg 552

<210> 74
<211> 450
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 74
tggaggcttc gagggaagtg aggttccctc ggacacccta gtgggaaggc tccacgcggt 60

aatggaacca cgctgtgaaa cctttgcctt tgggtgtcat ggtggaagca aatcttagaa 120

gacatttaat ttaaaaaatt cagttttaaa aaatgttgac ttaaaaagca gttttgaaaa 180

acaacctgga attagcctga gatcgatgcc aactcttagc agtctgtata ctaaacacag 240

ttaaacaact gtagctgctg gcaagctgga acctttttgt aaagaagcac ataaaaagga 300

cagaactggt ggaaggtgca ctggtctttc cacatcgcca ccaggcgttt tgaagcgtgc 360

tgctgacacg ctactcanat gcttctggaa gccaaacaat aanaaaaanc cccattgttt 420

cccttgctgg gttttacccn ccatggtgga 450

<210> 75
<211> 432
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 75
ggacaatgag gaggggtgc acgtggaatc cccacggata ggccggacgc cgggcaggag 60

cctttgcagg ggtgcacagc ctcctctgga agccctggtc gctgcctggt gcctgctgca 120

ccctgcgggc tccgcagcgg tggagccagg cctgaactgc ctgctcttgg ccccgcctgc 180

ggccctctgc cctttgtctt gcccgtgggg cccggggcct caagctggcc cggggttcct 240

gaagttagct gacgatgggc tggcctctgg ggctgggtcg tgggccttgt gcactggccg 300

ccacgtcacc agcgccaggc ctacccgcgg tgctgctgga gacgcgggat gcccgggctc 360

gggctgtgct ggatcccctg gcgctgcgaa ccccgtaccc ctttccaatc gcgggcncgg 420

nttaaagccc ga 432

<210> 76
<211> 501
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 76
gacgagacct agccggcncc atgcgcgcct tgagcctggc gaacagttcg gctggcgcga 60

cgcgcctgat gctcttcgtc cagatcatcc tgatcgacta gaccggcttc catccgagta 120

cgtgctcgct cgatgcgatg tttcgcttgg tggcgaatgg gcaggtagcc ggatcaagcg 180

tatcgagccg cccgattgca tcagccatga tggatacttt ctcggcagga gcaaggtggg 240

atgacaggag atcctgcccc ggcacttcgc ccaatagcag ccagtccctt cccgcttcag 300

tgacaacgtc gagcacagct gcccaaggaa cgcccgtcgt ggccagccac gatagccgcg 360

ctgcctcgtc ctgcagttca ttcagggcac cggacaggtc ggtcttgaca aaaagaaccg 420

ggcgcccctg ccgttgacag ccggaacacg gcggcatcag agcagccgat tgtctcgttg 480

tgcccagtca tagccgaatt c 501

<210> 77
<211> 826
<212> DNA
<213> Homo sapiens

<400> 77
gcgccctgtg gggatgacgc accatcctgt ttgtttgcac caagtcattt atctcgtgca 60

ccccaggggg ccgtggtccc tgccgggcca tcatgtctgc ttcccttatt tgggttttct 120

gccccctcac ttcatttctc acttcgcttt tcctccttat ccctttgcag tcttgctttt 180

gggggcattg ctcagccagt aatttgaggg acacctcgtg gagccctagt gtggagccgt 240

cagagcctgg gtaggattct ccgtggtgag gtgctcaggg agacacagga gcattccggc 300

gcctgttcct tgtgcacatc cgcaagtgtc tgcagtgaga ggcatgggtc ccatcttgaa 360

tgccaacaat gtggcaccca caccccactt gatggggccg agccacagct ggccaggttg 420

accaccatgg acgtgccaga ggcatccgaa acccagctct tgcccagctg ttccactgcc 480

aactccagcg ttagcaaagc agctctccct tgctttgtct tctacagcag agaacagatt 540

aaaagagaag ctgcaggcag agaaatgcct cttggagcca gatgccccaa aggatctctt 600

tgaacaaagg gttgctcagg tcagcgttag ttcctggcat caagcaacaa aatcagagat 660

gctaacagtt ctcagattca ctccaagtga agactcaaag ctggatttat aaatccccac 720

agagccgctg tgcagaggta gagggccggt ttcaggatga ggaagccctc ttggaagcac 780

cgtcctccgg ctaacaagcc tccaacctac tgtcggcagg gagaac 826

<210> 78
<211> 433
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 78
tgcgcagctc cgcgangtgc ccggcgggcc cgaccctcag actcgcttgt ccctggagac 60

caaccctagc gaccaggctc tgccggatcc cgtcgggttt caactcctat tccgaaggtc 120

ctttctcccc taatcacaac acccactcgc ctctttttcc tcctcttcct cagcttccac 180

cgccgaccgg gcagccccag ttacccgata acggctccca aggccccgtg tttacattct 240

ttcccactgg aagcagaaat tatcacgccc aaattcctac ctgccttccc tggattcctg 300

gtttcctaag aaacgggttt ggcccacccc tgggcgttcg aacagtccac agaagcgggc 360

aaaggaaaga cgactcagtc tttcccctcc gccaatctct tctccgggac cacagatccc 420

agaagtcacc gcg 433

<210> 79
<211> 424
<212> DNA
<213> Homo sapiens

<400> 79
ggcgggcccg accctcagac tcgcttgtcc ctggagacca accctagcga ccaggctctg 60

ccggatcccg tcgggtttca actcctattc cgaaggtcct ttctccccta atcacaacac 120

ccactcgcct ctttttcctc ctcttcctca gcttccaccg ccgaccgggc agccccagtt 180

acccgataac ggctcccaag gccccgtgtt tacattcttt cccactggaa gcagaaatta 240

tcacgcccaa attcctacct gccttccctg gattcctggt ttcctaagaa acgggtttgg 300

cccacccctg ggcgttcgaa cagtccacag aagcgggcaa aggaaagacg actcagtctt 360

tcccctccgc caatctcttc tccgggacca caaatcccag aagtcaccgc ggccgctaag 420

ccga 424

<210> 80
<211> 285
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 80
caaccggggg gcanaggcga tcaaaantgg ggtgcgctgt ggtgggcgac acgtgtggcg 60

cgggtctcat tatccgccct tttcacttcc tggactggaa atggcagacc atatgatggc 120

aatgaaccac gggcgcttcc ccgacggcac caatgggctg caccatcacc ctgcccaccg 180

catgggcatg gggcagttcc cgagccccca tcaccaccag cagcagcagc cccagcacgc 240

cttcaacgcc ctaatgggcg agcacataca ctacggcgcg ggcaa 285

<210> 81
<211> 401
<212> DNA
<213> Homo sapiens

<400> 81
cagatatgta tcctcctctt tccaaccctg cgtccctttg aggcctggtc ggcgttccca 60

acctgccccc accccaccaa cccctgtccc tttggccatt agtcccggat tatctagcga 120

tgccccgtgt accgtctggc tttgctgttt actccgcgct cggccagttg aggccttttg 180

tatttattcc tgattttctc ataggggtaa agtgccttcg ggaggatagg acaagtccca 240

tcctgttcat acgaattaca gctcggactt cgggcccttt tacactgcct tttgtatctg 300

ttaacttgcg ctaaaaacga ttcggttctt ttttttgagg aaggggggttg gggggcggag 360

actctgtcgc ccagtcctga gggccgcggc gcgcaagccg a 401

<210> 82
<211> 268
<212> DNA
<213> Homo sapiens

<400> 82
atagcgcgca caactgtgtc tcttacccag gcacatgcac tatccctgat cccggtgcat 60

gatgggaatg tagtcctgca gccctgtgac caaagggctg ggagtgttta tgagacagca 120

tctctcagca agcaaagcaa ggcctgcaca gccccgcctt ttcctccagt gaggcgcact 180

gttcattaag gagtgttcat gagattacat tttccatcaa gcccagccag tcacgcacag 240

ctctacctct tcctctgccg ccccgcaa 268

<210> 83
<211> 989
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 83
gggtaatggg ggtgaacaga gagggatgcc gaggccagct tgtagtgtgg ctgttggtct 60

tgtccatcct atggcacaac cctgtcacca cccagatttt gttaggagtc ctcccccaac 120

ttgagagtgg aagctccttt ggcacaaaaa ggggttctgc atcatccccc agcccccagc 180

cctgagcctg ggtctggctc tgaactagac ctccatgaat gaatgcacag catcagtggg 240

gatccaccat catggggaaa tagtagatac aggaatgatt ttccaaccag attacagact 300

atttcaagcc cagccagagc ctaccaggcc aacattcccc aggcttgtgc ctctccgagc 360

ctcagattgc tcatccttca aacgagggac agctctgctg gcattacctg aactctaggg 420

tcctttataa gctcagactc cagcttagag cacacattga gaggctgctg caccccagag 480

ccacatacgt gcaacagagg gtggtccaga ccccttattg gtccccatgg ggtttgagag 540

agaagcctcc agaccagctc aacttctccc tcatctcact taggcctttg cacccagctc 600

ttaggaggtt gtcaggtcac agtgccccat ttcttttctc ttccccagaa atcatgcggg 660

ggatacctgc tcagacagga ccttcatgaa agccaggctg tgaggtgtgt tggggaatgc 720

ataattgata ggccatcgtt cggaggccct cctggaggac caaaatgtaa tcagcagtgg 780

cgagcttgtt cacgacagga attcctttta catcctggtg aggccaaaga cctggcaagc 840

aagtccctct ggtcattaaa gaagcatcct gacttgangc aggncacctt aggtcactgc 900

agccacaaaa atctttgntg ctggattcna aagtaggcat tggggctggg atctgggctc 960

tggcatcctt gancgtgtcg ggggccaaa 989

<210> 84
<211> 250
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 84
cgggctcgaa acttcgaaga ccgcggaacc cgaagcngcn cttggctcna atcgcttcgg 60

ctcgaggcgc ccgtncgggt cacgtgaggt gggggcgggc cgaagagggg ggctcccctc 120

ctcctgccgc agggttggcc gcaagtgcgc ttcaagaggc gcttgatgac ggttaatgtt 180

gcagcccgga agatgacttt tttctcctcc ttgggttgcg gcaggccgtt agtgggaggt 240

cgcgtcccga 250

<210> 85
<211> 402
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 85
ttctcccttg tcatcccctt accagagcca cagaaattat ccctgtgggc tcccttgtcc 60

tcactcggcc ttttctggag ttaagagatc caagccaact actgggtctg ttccctgcta 120

aaatcttagg ccggcgtccc atccacccat ccccatgcct aggactttta agctggcaac 180

ggtacctggg tttagttttc ccttcgtata tcactatctt cgtngcttac cttcttgtgc 240

ctaaagttcc accgatgtgc aaggngatta accactaaag tgcacctgac actactcttg 300

acaaattgca gttgggaggt gagttgatga ctggccggta aatcaaaagt gcttatttag 360

ggagtgaggg ggcccgcggc anaagccgan ttccagcaca ct 402

<210> 86
<211> 595
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 86
gatcccagaa ggttctggag ccgagtatca gagtttgagc agcgagtcca gcctagcaga 60

agcgggtgtt gaccggagac ttttcaatgg tgcaaaatga cacactgctt ttgacttggg 120

gatctgtccc ttgtggcacc agaagctaca acaggtncac ctggattcca gctctagctg 180

gactcggtaa ttgctaagtg ccagctctga agtctgtgat tccgtggaaa tccctttcaa 240

gcccgaattc tgtttttttat gggcctcttg tccaaacagt ttgacttgtg aactctgttt 300

ctgtcaagtt gacacttggg cttggcaccc attcatgagc cagatgaaag cggctaaatg 360

cccgaaaaaa taaaggnttt tacctttttt ttgaaccatt ggtgagcatn taaaaaaatt 420

agggaaggta aaacccaacc nggncaaacc caactnaaca nttttttttt ccnaaacaag 480

ggggggctan tttttcactt ggaaaaacaa acaattttaa ttgantcttg ananggtgga 540

naaccaaaat tttttgttgg gttgggttcc gnagnccgaa ttntgcaaat ttctt 595

<210> 87
<211> 304
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 87
cgtggcccga tgcattcagg gagccctctg tgttggccgc atagcaggtg tagttgccgg 60

catcctggat gaagacgggc gcgatctgta gacccccccga ttcaagaagc atgaacctag 120

gaatccggac agagccactg gccagaatgt ggttttctaa agaacagtgg agaaaagagg 180

catgttacag tcgtaacgct tgaaggaaat gaagatagtg gttagagcca taagcaagta 240

atatggttcg gctccgtgtc cccacccaag tctcgtctng aattgcaatc cccacgtcgg 300

cgca 304

<210> 88
<211> 296
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 88
ggctttcgnt aggagttaat ggggcattgg ngggtgggat ggcagggctg ccagcatctg 60

acccaggagg ctgggaggag gctgctgtgt gaatacacgc tcggcctctc acagtggctg 120

ccgccgcatt agccccttgt gcttcaggga acagagcatc cgtgatggat gagactttaa 180

ttaaagtaat gagacattta taatcgcggt tatctccaaa attaggcctt ttagcaatta 240

ttcctgggga atattcctcc ggtagatagc tccctttttа gaacaacgtc ggcgca 296

<210> 89
<211> 220

<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 89
attggcccgn caggcgggaa acangctgnn nttctctnac cgttntccag cactgcccag 60

accaggaggc gcagggagag gaggggncag cggttccgng accgctcctc ccgctgtccc 120

tgctctccag cctntgcctc tgcaggagcc cgcgggantt gccccaggcc cctgtcccca 180

cctgtggctc ccgtcctggt cgctcccggg gccgcggcaa 220

<210> 90
<211> 273
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 90
gnagggnggn ggtcgcggac gccggtgggc agttcttgtt cggtgatgtg ggttaaaaag 60

gactgcagcg aggagccggg gcggcgctcg gagtaatcac cggcggcatc aaaaagcgcc 120

atcatggcat cgaggtcgcg gtctgcttgg gagccggtgg cgccgccgcg caaggcagat 180

gcctgcaggc gcatatccag ctcggtagcg ctccatacct cccacaggat ttcttccaca 240

gaggcttggg cttgtatagc ctgccgcccc gca 273

<210> 91
<211> 361
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> ()..()
<223> "n" refers to an undetermined base

<400> 91
acggcttctn tnctaagtga cacggtgtgt gaaattcggt tggggaggta gttctgtaaa 60

ctgcgtctcc ccgccagcta aggaagttga gtgaagggag cgttgccgtc tgggaatcgt 120

agtcctcaca aaggcgtgag taggcggcaa ataaggattt gggtttagcc ttggggattc 180

actcctgtca aagctgttag agaagctccc anaactcnta aagtaacaga aactacttgc 240

ggcaacattt gtaacttcca cctggctcat tatcttccac tgttaccttg tgttctagat 300

aagttataat ttattctaca tatcgttcag aagtcttgtg cctgttccat attgtnagca 360

t 361

<210> 92
<211> 462
<212> DNA
<213> Homo sapiens

<400> 92
gctgcccaca ctggatggga aggaccggcg cctgcagcat ctgccctcca agccttcgta 60

gctccctcct tcctgcagga taaactctaa actccttagc acaacgtggg agccttctca 120

gagactgggt ccaacccatc tccagccgca gcctcccctc ctggccccac tgccacaccc 180

ccgggcctcc ggccacactg agcctctccc ggtttcccag gatacaacac tcgcccattc 240

atagtgtggt gccttttgca cgtgctgttc ctctgcttgg ggatgctgtt ggtctttctc 300

agccaggtga agaggacgct gaatgtcacc tgcttgagta tcaggaccgg ggactgggcg 360

ctggacctag actcttggcc ctggagagaa gccctgcatg gggccgcagc ctgccccgt 420

ccctgctcac agaaaagctc agccttgcag ccgcgtggga ga 462

<210> 93
<211> 591
<212> DNA
<213> Homo sapiens

<400> 93
caaagtcacc tccacggtgc ggctcagcag ctcggcacac ttggtcatgg tgtcggggaa 60

ggcgccctcc agctgtaggt gggtagtggc agaacaggag ggtgagggga gagtccgaac 120

tgtccccact tggccgttcc ctccccactg gggggccctg agccagtggc ctcctctctc 180

ggggcctccc cggaaggagc caaggtctgt ctgcgaggca ccggtccccg gccacggcca 240

tcagccccca gaggtggatc agggcatcac ccccactcca cagctgaggc caggggtca 300

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aagacacgaa ggggaaggcg caagcttcta ccaagctcan tttgcccatc tggtgcccac 360

ctgcctngta tttggtgact tggaggatag gaagg 395

We claim:

1. A diagnostic or prognostic assay for cancer, comprising:

(a) obtaining a tissue sample from a test tissue;

(b) performing a methylation assay on DNA derived from the tissue sample, wherein the methylation assay determines the methylation state of a CpG dinucleotide within a DNA sequence of the DNA, and wherein the DNA sequence is a sequence selected from the group consisting of sequences of SEQ ID NOS:1-103, sequences having a nucleotide sequence at least 90% identical to sequences of SEQ ID NOS:1-103, CpG island sequences associated with sequences of SEQ ID NOS:1-103, CpG island sequences associated with sequences having a nucleotide sequence at least 90% identical to sequences of SEQ ID NOS:1-103, and combinations thereof, wherein the CpG island sequence associated with the sequence of the particular SEQ ID NO is that contiguous sequence of genomic DNA that encompasses at least one nucleotide of the particular SEQ ID NO sequence, and satisfies the criteria of having both a frequency of CpG dinucleotides corresponding to an Observed/Expected Ratio >0.6, and a GC Content >0.5; and

(c) determining a diagnosis or prognosis based, at least in part, upon the methylation state of the CpG dinucleotide within the DNA sequence.

2. The diagnostic or prognostic assay of claim 1 wherein the DNA sequence is a sequence selected from the group consisting of CpG island sequences associated with sequences of SEQ ID NOS:1-103, CpG island sequences associated with sequences having a nucleotide sequence at least 90% identical to sequences of SEQ ID NOS:1-103, and combinations thereof.

3. The diagnostic or prognostic assay of claim 2 wherein the DNA sequence is a sequence selected from the group consisting of CpG island sequences associated with sequences of SEQ ID NOS: 2, 4, 6, 7, 9-16, 19, 20, 22-33, 35-43, 48, 51-55, 59, 60, 64, 71, 76, 78-81, 84 and 87-90, and combinations thereof.

4. The diagnostic or prognostic assay of claim 1 wherein the methylation assay procedure is selected from the group consisting of MethyLight, MS-SNuPE, MSP MCA, COBRA, and combinations thereof.

5. The diagnostic or prognostic assay of claim 1 wherein the methylation state of the CpG dinucleotide within the DNA sequence is that of hypermethylation, hypomethylation or normal methylation.

6. The diagnostic or prognostic assay of claim 1 wherein the cancer is selected from the group consisting of bladder cancer, prostate cancer, colon cancer, lung cancer, renal cancer, leukemia, breast cancer, uterine cancer, astrocytoma, glioblastoma, and neuroblastoma.

7. A kit useful for the detection of a methylated CpG-containing nucleic acid comprising a carrier means containing one or more containers comprising:

(a) a container containing a probe or primer which hybridizes to any region of a sequence selected from the group consisting of SEQ ID NOS:1-103, and sequences having a nucleotide sequence at least 90% identical to sequences of SEQ ID NOS:1-103; and

(b) additional standard methylation assay reagents required to affect detection of methylated CpG-containing nucleic acid based, at least in part, on the probe or primer.

8. The kit of claim 7, wherein the additional standard methylation assay reagents are standard reagents for performing a methylation assay from the group consisting of MethyLight, MS-SNuPE, MSP MCA, COBRA, and combinations thereof.

9. The kit of claim 7, wherein the probe or primer comprises at least about 12 to 15 nucleotides of a sequence selected from the group consisting of SEQ ID NOS:1-103, and sequences having a nucleotide sequence at least 90% identical to sequences of SEQ ID NOS:1-103.

10. An isolated nucleic acid molecule comprising a methylated or unmethylated polynucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:97, and SEQ ID NO:100.

11. The nucleic acid of claim 10, wherein the nucleic acid is methylated.

12. The nucleic acid of claim 10, wherein the nucleic acid is unmethylated.

Abstract

There is disclosed 103 novel methylation-altered DNA sequences ("marker sequences") that have distinct methylation patterns in cancer, compared to normal tissue. In many instances, these marker sequences represent novel sequences not found in the GenBank data base, and none of these marker sequences have previously been characterized with respect to their methylation pattern in human cancers including, but not limited to those of bladder and prostate. These 103 sequences have utility as diagnostic, prognostic and therapeutic markers in the treatment of human cancer, and as reagents in kits for detecting methylated CpG-containing nucleic acids.